FORM PTO-1390 (Rev 5-93) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **PATKRI P02AUS**

U.S. APPLICATION NO. (If known, see 37 C.F.R.): **09/889326**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/00084	**January 7, 2000**	**January 18, 1999**

TITLE OF INVENTION

METHOD FOR ELECTROCHEMICALLY DETECTING NUCLEIC ACID-OLIGOMER HYBRIDISATION EVENTS

APPLICANT(S) FOR DO/EO/US

Gerhard HARTWICH

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ■ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ■ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ■ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ■ has been transmitted by the International Bureau. (PCT/IB/308 mailed **20 July 2000**)
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ■ A translation of the International Application into English (35 U.S.C. 371(c)(2)) is attached.
7. ■ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ■ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. ■ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ■ An Information Disclosure Statement under 37 CFR 1.97 and 1.98 with PTO FORM 1449.
12. ■ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ■ Other items or information:
    - ■ Preliminary Examination Report
    - ■ Annexes to Pre. Ex. Rep.
    - ■ International Search Report
    - ☐ German Novelty Search Report
    - ■ 51 copies of citations
    - ■ Form PCT/IB/308
    - ■ International Publ. No. **WO 00/42217** (Face page only)
    - ■ Copy of Request
    - ■ 6 sheets of formal drawings
    - ■ Abstract
    - ■ German Language Specification
    - ■ Submission of Formal Drawings
    - ■ Marked-Up Version of Amended Specification

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Transmittal Letter and the papers indicated as being transmitted therewith is being deposited with the United States Postal Service on this date **July 11, 2001** in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number **EL469354975US** addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Michael J. Bujold
(typed or printed name of person mailing paper)

(signature of person mailing paper)

17. ■ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO $860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)). $710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	860	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	0	

Claims	Number Filed	Number Extra	Rate		
Total Claims	71 - 20 =	51	x $18.00	6978	
Independent Claims	1 - 3 =	0	x $80.00		
Multiple dependent claim(s) (if applicable)			+ $270.00	0	
			TOTAL OF ABOVE CALCULATIONS =	1838	
Reduction by 1/2 for filing by small entity, if applicable. **Applicant Claims Small Entity Status.** (Note 37 CFR 1.9, 1.27, 1.28).				919	
			SUBTOTAL =	919	
Processing fee of $130.00 for furnishing the English translation later the ☐ 20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	0	
			TOTAL NATIONAL FEE =	0	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property			+	40	
			TOTAL FEES ENCLOSED =	959	
				Amount to be: refunded	$
				charged	$

a. ■ A check in the amount of $ **959.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 04-0213 in the amount of $____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ■ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 04-0213. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Michael J. Bujold -- Registration No. 32,018
Davis & Bujold, P.L.L.C.
Fourth Floor
500 North Commercial Street
Manchester, NH 03101-1151
Telephone (603) 624-9220
Telefax (603) 624-9229

#4/B

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	Gerhard HARTWICH
Serial no.	:	09/889,326
Filed	:	with an effective filing date of January 7, 2000
For	:	METHOD FOR ELECTROCHEMICALLY DETECTING NUCLEIC ACID-OLIGOMER HYBRIDISATION EVENTS
Group Art Unit	:	
Examiner	:	
Docket	:	PATKRI P02AUS

The Commissioner of Patents and Trademarks
Washington, D.C. 20231

RESPONSE TO NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 AND AMENDMENT TO INCORPORATE SEQUENCE LISTING

Dear Sir:

[XXX] NO FEES ARE PAYABLE WITH RESPECT TO THIS RESPONSE.

In response to the Notification of Missing Requirements under 35 U.S.C. § 371 (referred to herein as NOTIFICATION) mailed September 5, 2001, please enter the following before reconsideration of this application.

In the Drawings:

Please amend Fig. 1 of the drawings presently on file per the attached Submission. The Applicant respectfully requests deferment of formal entry of such drawing amendments until such time as the Examiner approves the requested drawing amendment(s).

In the Specification:

Please cancel paragraphs 9, 20, 96, 98, 112 and 118 of the specification, in their entirety, in favor of a clean form of paragraphs 9, 20, 96, 98, 112 and 118 of the specification, without any markings thereon, as follows. Also accompanying this response is a copy of the original paragraphs of the specification which show the addition(s) (by underlining and bold) and the deletion(s) (by strikeout) to the canceled specification paragraphs. Please enter the replacement specification paragraphs into the record of this case.

Secondly, after page 66 of the specification, please insert the attached Sequence Listing and renumber the claims pages to begin with page 72.

[CLEAN FORM OF PARAGRAPH 9]

[009] Some years ago, a further method of DNA sequencing was developed on the basis of optical (or autoradiographical) detection, namely sequencing by oligomer hybridization (cf. e.g. Drmanac et al., Genomics 4, (1989), pp. 114-128 or Bains et al., Theor. Biol. 135, (1988), pp. 303-307). In this method, a complete set of short oligonucleotides or nucleic acid oligomers (probe oligonucleotides), e.g. all 65,536 possible combinations of bases A, T, C, and G of an oligonucleotide octamer, are bound to a support material. The attachment occurs in an ordered grid comprising 65,536 test sites, each rather large amount of an oligonucleotide combination defining one test site, and the position of each individual test site (oligonucleotide combination) being known. On such a hybridization matrix, the oligomer chip, a DNA fragment whose sequence is to be determined (the target) is labeled with fluorescent dye (or ^{32}P) and hybridized under conditions that allow only one specific double-strand formation. In this way, the target DNA fragment binds only to those nucleic acid oligomers (in this example to the octamers) whose complementary sequence corresponds exactly to a portion (an octamer) of its own sequence. Thus, all of the nucleic acid oligomer sequences (octamer sequences) present in the fragment are determined by optical (or autoradiographical) detection of the binding position of the hybridized DNA fragment. Due to the overlapping of neighboring nucleic acid oligomer sequences, the sequential sequence of the DNA fragment can be determined using appropriate mathematical algorithms. One of the advantages of this method lies in the miniaturization of the sequencing and thus in the enormous amount of data that can be simultaneously captured in one operation. In addition, primer and gel-electrophoretic separation of the DNA fragments can be dispensed with. This principle is exemplified in Figure 1 for a 13-base-long DNA fragment (SEQ ID NOS: 2-18).

[CLEAN FORM OF PARAGRAPH 20]

[020] ***Modified Surfaces/Electrodes***

mica	Muscovite lamina, a support material for the application of thin films.
Au-S-$(CH_2)_2$-ss-oligo-spacer-UQ(RC)	Gold film on mica having a covalently applied monolayer of derivatized 12-bp single-strand DNA oligonucleotide (sequence (SEQ. ID. No.: 1): TAGTCGGAAGCA). Here, the oligonucleotide's terminal phosphate group at the 3'-end is esterified with $(HO-(CH_2)_2-S)_2$ to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH, the S-S bond being homolytically cleaved and producing one Au-S-R bond each. The terminal thymine base at the 5'-end of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH_2-CH_2-NH_2, this residue, in turn, being joined via its free amino group with the carboxylic-acid group of the modified ubiquinone-50 by amidation. Thereafter, the UQ is reconstituted with the remaining RC.
Au-S-$(CH_2)_2$-ds-oligo-spacer-UQ(RC)	*Au-S-$(CH_2)_2$-ss-oligo-spacer-UQ(RC)* hybridized with the oligonucleotide that is complementary to the ss-oligo (sequence (SEQ. ID. No.: 1): TAGTCGGAAGCA).
Au-S-$(CH_2)_2$-ss-oligo-spacer-Q-ZnBChl	Identical to *Au-S-$(CH_2)_2$-ss-oligo-spacer-UQ(RC)* with the exception that, instead of the RC attached via UQ, Q-ZnBChl is attached as the photoinducibly redox-active moiety.
Au-S-$(CH_2)_2$-ds-oligo-spacer-Q-ZnBChl	*Au-S-$(CH_2)_2$-ss-oligo-spacer-Q-ZnBChl* hybridized with the oligonucleotide that is complementary to the ss-oligo (sequence (SEQ. ID. No.: 1): TAGTCGGAAGCA).

[CLEAN FORM OF PARAGRAPH 96]

[096] Fig. 4 Shows a detailed schematic diagram of the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-Q-ZnBChl of Figure 3 having gold as the solid support material, mercaptoethanol as the spacer (-S-CH_2CH_2- spacer) between the electrode and the oligonucleotide, and -CH_2-CH=CH-CO-NH-CH_2-CH_2-NH- as the spacer between the electron acceptor PQQ and the oligonucleotide, as well as a diagram of the sequence of the photoinduced electron transfer steps. The apoprotein of the RC is indicated only as a shell (solid line) (cf. Structure 1). The 12-bp probe oligonucleotide of the exemplary sequence (SEQ ID NO: 1) 5'-TAGTCGGAAGCA-3' in the hybridized state is shown in detail;

[CLEAN FORM OF PARAGRAPH 98]

[098] Fig. 6 Shows a detailed schematic diagram of the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-Q-ZnBChl of Figure 5 having gold as the solid support material, mercaptoethanol as the spacer (-S-CH_2CH_2- spacer) between the electrode and the oligonucleotide, and -CH_2-CH=CH-CO-NH-CH_2-CH_2-NH- as the spacer between the electron acceptor PQQ and the oligonucleotide, as well as a diagram of the sequence of the photoinduced electron transfer steps. The 12-bp probe oligonucleotide of the exemplary sequence (SEQ ID NO: 1) 5'-TAGTCGGAAGCA-3' in the hybridized state is shown in detail.

[CLEAN FORM OF PARAGRAPH 112]

[112] For incubation, a doubly modified 12-bp single-strand oligonucleotide having the sequence (SEQ ID NO: 1) 5'-TAGTCGGAAGCA-3' was used, which is esterified with (HO-$(CH_2)_2$-$S)_2$ at the phosphate group of the 3'-end to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH. At the 5'-end, the terminal thymine base of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH_2-CH_2-NH_2. Approximately 10^{-4} to 10^{-1} molar 2-hydroxy-mercaptoethanol (or another thiol or disulfide linker having a suitable chain length) was added to a $2x10^{-4}$ molar solution of this oligonucleotide in HEPES buffer (0.1 molar in water, pH 7.5 with 0.7 molar addition of TEATFB, see abbreviations) and the gold surface of a test site was completely wetted and incubated for 2-24 hours. During this reaction time, the disulfide spacer P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH of the oligonucleotide is homolytically cleaved. In this process, the spacer forms a covalent Au-S bond with Au atoms of the

surface, thus causing a 1:1 coadsorption of the ss-oligonucleotide and the cleaved 2-hydroxy-mercaptoethanol. The free 2-hydroxy-mercaptoethanol that is also present in the incubation solution is likewise coadsorbed by forming an Au-S bond (incubation step).

[CLEAN FORM OF PARAGRAPH 118]

[118] For incubation, a doubly modified 12-bp single-strand oligonucleotide having the sequence (SEQ ID NO: 1) 5'-TAGTCGGAAGCA-3' was used, which is esterified with $(HO\text{-}(CH_2)_2\text{-}S)_2$ at the phosphate group of the 3'-end to form $P\text{-}O\text{-}(CH_2)_2\text{-}S\text{-}S\text{-}(CH_2)_2\text{-}OH$. At the 5'-end, the terminal thymine base of the oligonucleotide is modified at the C-5 carbon with $-CH{=}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}NH_2$. A $2x10^{-4}$ molar solution of this oligonucleotide in the hybridization buffer (10 mM Tris, 1 mM EDTA, pH 7.5 with 0.7 molar addition of TEATFB, see abbreviations) was hybridized with a $2x10^{-4}$ molar solution of the (unmodified) complementary strand in the hybridization buffer at room temperature for approx. 2 hours (hybridization step). After hybridization, approx. 10^{-4} to 10^{-1} molar 2-hydroxy-mercaptoethanol (or another thiol or disulfide linker having a suitable chain length) was added to the now $1x10^{-4}$ molar double-strand oligonucleotide solution and the gold surface of a test site was completely wetted and incubated for 2 – 24 hours. During this reaction time, the disulfide spacer $P\text{-}O\text{-}(CH_2)_2\text{-}S\text{-}S\text{-}(CH_2)_2\text{-}OH$ of the oligonucleotide is homolytically cleaved. In this process, the spacer forms a covalent Au-S bond with Au atoms of the surface, thus causing a 1:1 coadsorption of the ds-oligonucleotide and the cleaved 2-hydroxy-mercaptoethanol. The free 2-hydroxy-mercaptoethanol that is also present in the incubation solution is likewise coadsorbed by forming an Au-S bond (incubation step).

REMARKS

In response to the Notification, the drawings are suitably amended and the required Sequence Listing is attached. Also transmitted herewith is a copy of the Sequence Listing in computer readable form. As required by 37 CFR 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d), the Applicants' Attorney hereby states that the content of the Sequence Listing, in the attached paper form and computer readable form of the Sequence Listing, are the same and the submission does not include any new matter.

The Applicant respectfully requests that any outstanding objection(s) or requirement(s), as to the form of this application, be held in abeyance until allowable subject matter is indicated for this case.

In the event that there are any fee deficiencies or additional fees are payable, please charge the same or credit any overpayment to our Deposit Account (Account No. 04-0213).

Respectfully submitted,

Michael J. Bujold, Reg. No. 32,018
Customer No. 020210
Davis & Bujold, P.L.L.C.
Fourth Floor
500 North Commercial Street
Manchester NH 03101-1151
Telephone 603-624-9220
Facsimile 603-624-9229
E-mail: patent@davisandbujold.com

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service, with sufficient postage, as First Class Mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on November 5, 2001 .

By: _____

Print Name: Michael J. Bujold

[AMENDMENTS MADE TO PARAGRAPH 9]

[009] Some years ago, a further method of DNA sequencing was developed on the basis of optical (or autoradiographical) detection, namely sequencing by oligomer hybridization (cf. e.g. Drmanac et al., Genomics 4, (1989), pp. 114-128 or Bains et al., Theor. Biol. 135, (1988), pp. 303-307). In this method, a complete set of short oligonucleotides or nucleic acid oligomers (probe oligonucleotides), e.g. all 65,536 possible combinations of bases A, T, C, and G of an oligonucleotide octamer, are bound to a support material. The attachment occurs in an ordered grid comprising 65,536 test sites, each rather large amount of an oligonucleotide combination defining one test site, and the position of each individual test site (oligonucleotide combination) being known. On such a hybridization matrix, the oligomer chip, a DNA fragment whose sequence is to be determined (the target) is labeled with fluorescent dye (or ^{32}P) and hybridized under conditions that allow only one specific double-strand formation. In this way, the target DNA fragment binds only to those nucleic acid oligomers (in this example to the octamers) whose complementary sequence corresponds exactly to a portion (an octamer) of its own sequence. Thus, all of the nucleic acid oligomer sequences (octamer sequences) present in the fragment are determined by optical (or autoradiographical) detection of the binding position of the hybridized DNA fragment. Due to the overlapping of neighboring nucleic acid oligomer sequences, the sequential sequence of the DNA fragment can be determined using appropriate mathematical algorithms. One of the advantages of this method lies in the miniaturization of the sequencing and thus in the enormous amount of data that can be simultaneously captured in one operation. In addition, primer and gel-electrophoretic separation of the DNA fragments can be dispensed with. This principle is exemplified in Figure 1 for a 13-base-long DNA fragment **(SEQ ID NOS: 2-18)**.

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SEQUENCE LISTING

<110> HARTWICH, GERHARD

<120> METHOD FOR ELECTROCHEMICALLY DETECTING NUCLEIC ACID-OLIGOMER HYBRIDIZATION EVENTS

<130> 0163-2003

<140> 09/889,326
<141> 2000-01-07

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<220>
<223> Description of Artificial Sequence: synthetic oligonucleotide

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11/5/01 PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	Gerhard HARTWICH
Serial no.	:	09/889,326
Filed	:	with an effective filing date of January 7, 2000
For	:	METHOD FOR ELECTROCHEMICALLY DETECTING NUCLEIC ACID-OLIGOMER HYBRIDISATION EVENTS
Group Art Unit	:	
Examiner	:	
Docket	:	PATKRI P02AUS

The Commissioner of Patents and Trademarks
Washington, D.C. 20231

SUBMISSION OF PROPOSED DRAWING AMENDMENT FOR APPROVAL BY EXAMINER (37 CFR 1.123)

Dear Sir:

Attached hereto please find a copy of Fig. 1 of the original drawings with red ink markings showing proposed changes to the drawing(s) of this application for which the approval of the Examiner is requested.

In the event that there are any fee deficiencies or additional fees are payable, please charge the same or credit any overpayment to our Deposit Account (Account No. 04-0213).

Respectfully submitted,

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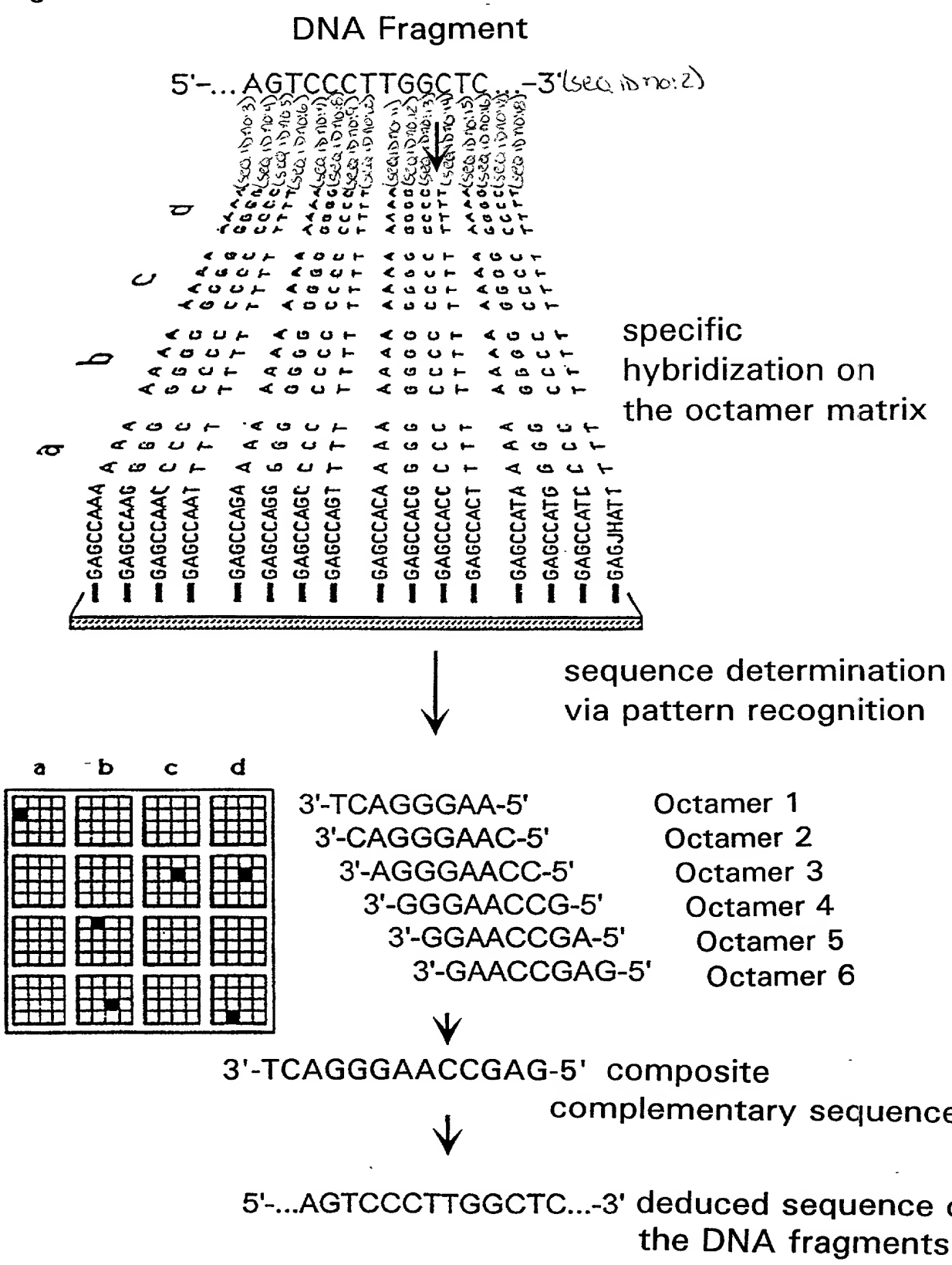
CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service, with sufficient postage, as First Class Mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on November 5, 2001 .

By: _____

Print Name: Michael J. Bujold

Fig. 1


DNA Fragment
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specific hybridization on the octamer matrix
sequence determination via pattern recognition
a b c d
3'-TCAGGGAA-5' Octamer 1
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3'-AGGGAACC-5' Octamer 3
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3'-GGAACCGA-5' Octamer 5
3'-GAACCGAG-5' Octamer 6
3'-TCAGGGAACCGAG-5' composite complementary sequence
5'-...AGTCCCTTGGCTC...-3' deduced sequence of the DNA fragments

07/11/01 PATENT APPLICATION #3/a

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	Gerhard HARTWICH
Serial no.	:	
For	:	METHOD FOR ELECTROCHEMICALLY DETECTING NUCLEIC ACID-OLIGOMER HYBRIDISATION EVENTS
Docket	:	PATKRI P02AUS

BOX PCT
The Commissioner of Patents and Trademarks
Washington, D.C. 20231

FIRST PRELIMINARY AMENDMENT

Dear Sir:

By way of preliminary amendment, please amend the above identified application as set forth below.

In the Specification:

Please cancel paragraphs 13, 15, 55, 72, 77, 80, 90 and 99 of the specification, in their entirety, in favor of a clean form of paragraphs 13, 15, 55, 72, 77, 80, 90 and 99 of the specification, without any markings thereon, as follows. Also accompanying this response is a copy of the original paragraphs of the specification which show the addition(s) (by underlining, shading and bold) and the deletion(s) (by strikeout) to the canceled specification paragraphs. Please enter the replacement specification paragraphs into the record of this case.

In the Claims:

Please cancel original claims 1-55, as well as any Chapter II amended claims, in favor of new claims 56-110 as follows.

[013] DETAILED DESCRIPTION OF THE INVENTION

[015] According to the present invention, this object is solved by the modified nucleic acid oligomer, the method of producing a modified nucleic acid oligomer, the modified conductive surface, the method of producing a modified conductive surface, and a method of electrochemically detecting nucleic acid oligomer hybridization events.

[055] According to the present invention, a redox-active moiety is covalently bound to a nucleic acid oligomer by the reaction of the nucleic acid oligomer with the redox-active moiety or portions thereof (see also the section "Detailed Description of the Invention"). This bond can be achieved in four different ways:

[072] Binding the nucleic acid oligomer to the conductive surface may take place before or after the redox-active moiety is attached to the nucleic acid oligomer. In the case of a redox-active protein/enzyme comprising apoprotein and cofactor(s), instead of the complete redox-active moiety, it is also possible for only the apoprotein, the apoprotein and a portion of the cofactors, or one or more of the cofactors to be attached and the redox-active moiety will be completed by subsequent reconstitution with the remaining missing portions. If a linked (at least bimolecular) electron-donor/electron-acceptor complex is used as the redox-active moiety, the electron acceptor (or donor) may, as described under b) or c) in the section "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," be attached to a terminal base, or in place of a terminal base, to the nucleic acid oligomer, and the electron donor (or acceptor) may be attached by subsequent covalent attachment to a reactive group of the electron acceptor (or donor) or, as described under a) in the section "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," by subsequent attachment to a terminal reactive group of the nucleic acid oligomer backbone at the same end (see also the section "Detailed Description of the Invention"). Alternatively, binding the nucleic acid oligomer to the conductive surface may take place before or after the spacer having a reactive group for binding the redox-active moiety is attached. Binding the already modified nucleic acid oligomer to the conductive surface, i.e. binding to the surface after the redox-active moiety is attached to the nucleic acid oligomer or after portions of the redox-active moiety are attached, or after the spacer having a reactive group for binding the redox-active moiety is attached, likewise takes place as described under a) to c) in this section.

[077] Regarding the individual steps in "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," as well as in "Binding an Oligonucleotide to the Conductive Surface," it should be noted that, in the section "Detailed Description of the Invention," the various combination possibilities of the individual steps that lead to the same end result are demonstrated in an example (Figure 2).

[080] The modification of the probe nucleic acid oligomers with a redox-active moiety may take place completely or in components of the redox-active moiety, either before or after the oligonucleotide probe is bound to the conductive surface. The various combination possibilities of the individual steps (reaction sequences) are demonstrated in the section "Detailed Description of the Invention" with the aid of Figure 2 using the example of a redox-active moiety bound to an electrode via a probe oligonucleotide.

[090] If the redox-active moiety is a photoinducibly redox-active moiety, the redox activity of the moiety is triggered only by light of a specific or any given wavelength. According to the present invention, this property is used to advantage in that electrochemical detection is triggered only by radiating light onto the surface hybrid having the general structure elec-spacer-ds-oligo-spacer-moiety (surface hybrid with hybridized target) and is maintained, at most, as long as light irradiation continues. Thus, particularly in the case of amperometric detection, if a photoinducibly redox-active moiety is used, under certain external conditions, (rather long-lasting) current will flow only if light is radiated onto the surface hybrid. Such external conditions are for example the presence of a reducing agent (or oxidizing agent) suitable for reducing (or oxidizing) a photoinductively-formed oxidized donor D^+ (or reduced acceptor A^-) of the photoinducibly redox-active moiety, and applying to the electrode a potential at which a photoinductively-formed reduced acceptor A^- (or oxidized donor D^+) of the photoinducibly redox-active moiety can be oxidized (or reduced), but the non-reduced acceptor A (or the non-oxidized donor D) cannot be oxidized (or reduced). In the section "Detailed Description of the Invention," this is explained in greater detail using various examples of an elec-spacer-ss-oligo-spacer-moiety having a photoinducibly redox-active moiety. In this way, detection using a photoinducibly redox-active moiety can be spatially limited to a certain test site or group of test sites of the oligomer chip by restricting the light to this test site or group of test sites. According to the present invention, various test sites (nucleic acid oligomer combinations) of an oligomer chip can thus be applied to a shared, continuous, electrically-conductive surface. A particular test site or

group of test sites can be addressed and amperometrically detected simply by applying a suitable external potential to the (entire) surface if precisely this test site or group of test sites is irradiated with light. The various test sites thus need not be applied to individual (micro-) electrodes that are electrically isolated from one another and individually controllable for applying a potential and reading out the current. Moreover, if surface hybrids having the general structure elec-spacer-ss-oligo-spacer-moiety are used with a photoinducibly redox-active moiety and amperometric detection, the read-out process for detecting individual sequence-specific hybridization events on the oligomer chip can be optimized by first reading out the test sites by roughly scanning them with appropriately focused light and then successively increasing the resolution capacity in the grids having hybridization events, so for example, for an octamer chip having 65,536 test sites, e.g. 64 groups of 1024 test sites each are read out, then the test site groups that are shown by amperometric measurements to exhibit hybridization events can be tested e.g. in 32 groups of 32 test sites each, and thereafter, in the test site groups that again exhibit hybridization events, the test sites are assayed individually. In this way, the individual hybridization events can be quickly assigned to specific probe oligomers with little experimental outlay.

[099] DETAILED DESCRIPTION OF THE INVENTION

56. (NEW) A nucleic acid oligomer modified by covalently attaching a redox-active moiety, wherein the redox-active moiety comprises at least one electron-donor molecule and at least one electron-acceptor molecule, the electron-donor molecule and electron-acceptor molecule not being joined with one another by nucleic acid oligomers.

57. (NEW) The modified nucleic acid oligomer according to claim 56, wherein the redox-active moiety comprises at least one redox-active, linked, to at least one bimolecular electron-donor/electron-acceptor complex, at least one electron-donor molecule of the redox-active moiety and at least one electron-acceptor molecule of the redox-active moiety being joined with one another via one or more bonds.

58. (NEW) The modified nucleic acid oligomer according to claim 57, wherein the bonds are covalent bonds.

59. (NEW) The modified nucleic acid oligomer according to claim 56, wherein the redox-active moiety comprises at least one redox-active, linked, to at least one bimolecular electron-donor/electron-acceptor complex, at least one electron-donor molecule of the redox-active moiety and at least one electron-acceptor molecule of the redox-active moiety being covalently joined via one or more branched or linear molecular moieties of any composition and chain length.

60. (NEW) The modified nucleic acid oligomer according to claim 59, wherein the branched or linear molecular moieties have a chain length of 1 - 20 atoms.

61. (NEW) The modified nucleic acid oligomer according to claim 59, wherein the branched or linear molecular moieties have a chain length of 1-14 atoms.

62. (NEW) The modified nucleic acid oligomer according to claim 56, wherein the redox-active moiety additionally comprises one or more macromolecules.

63. (NEW) The modified nucleic acid oligomer according to claim 56, wherein the redox-active moiety is the native or modified reaction center of photosynthesizing organisms.

64. (NEW) The modified nucleic acid oligomer according to claim 63, wherein the redox-active moiety is the native or modified reaction center of photosynthesizing bacteria.

65. (NEW) The modified nucleic acid oligomer according to claim 56, wherein at least one of the electron-donor molecules and electron-acceptor molecules is a pigment.

66. (NEW) The modified nucleic acid oligomer according to claim 65, wherein the pigment is a flavin, a (metallo)porphyrin, a (metallo)chlorophyll, a (metallo)bacteriochlorophyll, or a derivative of these pigments.

67. (NEW) The modified nucleic acid oligomer according to claim 56, wherein at least one of the electron-donor molecules and electron-acceptor molecules is a nicotinamide or a quinone.

68. (NEW) The modified nucleic acid oligomer according to claim 67, wherein the quinone is a pyrroloquinoline quinone (PQQ), a 1,2-benzoquinone, a 1,4-benzoquinone, a 1,2-naphthoquinone, a 1,4-naphthoquinone, a 9,10-anthraquinone, or one of their derivatives.

69. (NEW) The modified nucleic acid oligomer according to claim 56, wherein at least one of the electron-donor molecules and electron-acceptor molecules is a charge transfer complex.

70. (NEW) The modified nucleic acid oligomer according to claim 69, wherein the charge transfer complex is a transition metal complex.

71. (NEW) The modified nucleic acid oligomer according to claim 70, wherein the charge transfer complex is a Ru(II), Cr(III), Fe(II), Os(II), or Co(II) complex.

72. (NEW) The modified nucleic acid oligomer according to claim 56, wherein the modified nucleic acid oligomer can sequence-specifically bind single-strand DNA, RNA, and/or PNA.

73. (NEW) The modified nucleic acid oligomer according to claim 72, wherein the modified nucleic acid oligomer is a deoxyribonucleic acid oligomer, a ribonucleic acid oligomer, or a peptide nucleic acid oligomer.

74. (NEW) The modified nucleic acid oligomer according to claim 56, wherein the redox-active moiety is covalently bound to one of the phosphoric-acid groups, to one of the carboxylic-acid groups, to one of the amine groups, or to a sugar of the nucleic acid oligomer backbone.

75. (NEW) The modified nucleic acid oligomer according to claim 56, wherein the redox-active moiety is covalently bound to a sugar-hydroxy group of the nucleic acid oligomer backbone.

76. (NEW) The modified nucleic acid oligomer according to claim 56, wherein the redox-active moiety is covalently attached to a thiol group, a hydroxyl group, a carboxylic-acid group, or an amine group of a modified base of the nucleic acid oligomer.

77. (NEW) The modified nucleic acid oligomer according to claim 76, wherein the reactive thiol, hydroxyl, carboxylic-acid, or amine group of the base is covalently bound to the base via a branched or linear molecular moiety of any composition and chain length, the shortest continuous link between the thiol, hydroxyl, carboxylic-acid, or amine group and the base being a branched or linear molecular moiety having a chain length of 1-20 atoms.

78. (NEW) The modified nucleic acid oligomer according to claim 77, wherein the shortest continuous link between the thiol, hydroxyl, carboxylic-acid, or amine group and the base is a branched or linear molecular moiety having a chain length of 1-14 atoms.

79. (NEW) The modified nucleic acid oligomer according to claim 74, wherein the redox-active moiety is attached to an end of the nucleic acid oligomer backbone or to a terminal modified base..

80. (NEW) The modified nucleic acid oligomer according to claim 56, wherein the redox-active moiety is photoinducibly redox-active moiety..

81. (NEW) The modified nucleic acid oligomer according to claim 56, wherein, redox-active moiety is a chemically-inducibly redox-active moiety.

82. (NEW) The modified nucleic acid oligomer according to claim 56, wherein multiple redox-active moieties are attached to the nucleic acid oligomer.

83. (NEW) The method of producing a modified nucleic acid oligomer according to claim 56, wherein a redox-active moiety is covalently attached to a nucleic acid oligomer.

84. (NEW) The method of producing a modified nucleic acid oligomer according to claim 83, wherein the redox-active moiety is attached to a nucleic acid oligomer by covalently attaching at least one electron-donor molecule.

85. (NEW) The method of producing a modified nucleic acid oligomer according to claim 83, wherein the redox-active moiety is attached to a nucleic acid oligomer by covalently attaching at least one electron-acceptor molecule.

86. (NEW) The method of producing a modified nucleic acid oligomer according to claim 83, wherein the redox-active moiety is attached to a nucleic acid oligomer by covalently attaching at least one macromolecule or by covalently attaching at least one protein.

87. (NEW) The method of producing a modified nucleic acid oligomer according to claim 84, wherein the redox-active moiety is completed by adding at least one component selected from the group consisting of electron-acceptor molecules, electron-donor molecules, macromolecules, and proteins.

88. (NEW) The method of producing a modified nucleic acid oligomer according to claim 83, wherein the nucleic acid oligomer is bound to the redox-active moiety by one or more amidations with amine or acid groups of the redox-active moiety, by one or more esterifications with alcohol or acid groups of the redox-active moiety, by thioester formation with thioalcohol or acid groups of the redox-active moiety, or by condensation of one or more amine groups of the nucleic acid oligomer with aldehyde groups of the redox-active moiety and subsequent reduction of the resultant carbon-nitrogen double bond.

89. (NEW) The method of producing a modified nucleic acid oligomer according to claim 88, wherein at least one branched or linear molecular moiety of any composition and chain length is covalently attached to the redox-active moiety and the branched or linear molecular moiety has a reactive amine, hydroxyl, thiol, acid, or aldehyde group for covalent attachment to a nucleic acid oligomer.

90. (NEW) The method of producing a modified nucleic oligomer according to claim 89 wherein the shortest continuous link between the nucleic acid oligomer and the redox-active moiety is a branched or linear molecular moiety having a chain length of 1-20 atoms.

91. (NEW) The method of producing a modified nucleic acid oligomer according to claim 90, wherein the shortest continuous link between the nucleic acid oligomer and the redox-active moiety is a branched or linear molecular moiety having a chain length of 1-14 atoms.

92. (NEW) The modified conductive surface, wherein at least one type of modified nucleic acid oligomer according to claim 56, is attached to a conductive surface.

93. (NEW) The modified conductive surface according to claim 92, wherein the surface consists of a metal or a metal alloy.

94. (NEW) The modified conductive surface according to claim 93, wherein the surface consists of a metal selected from the group of metals consisting of platinum, palladium, gold, cadmium, mercury, nickel, zinc, carbon, silver, copper, iron, lead, aluminum, and manganese.

95. (NEW) The modified conductive surface according to claim 92, wherein the surface consists of a semiconductor.

96. (NEW) The modified conductive surface according to claim 92, wherein the surface consists of a semiconductor selected from the group comprising carbon, silicon, germanium, and tin.

97. (NEW) The modified conductive surface according to claim 84, wherein the surface consists of a binary compound of the elements of groups 14 and 16, a binary compound of the elements of groups 13 and 15, a binary compound of the elements of groups 15 and 16, or a binary compound of the elements of groups 11 and 17.

98. (NEW) The modified conductive surface according to claim 97, wherein the surface consists of a Cu(i) halide or an Ag(i) halide.

99. (NEW) A modified conductive surface according to claim 92, wherein the surface consists of a ternary compound of the elements of groups 11, 13, and 16, or a ternary compound of the elements of groups 12, 13, and 16.

100. (NEW) The modified conductive surface according to claim 92, wherein the attachment of the modified nucleic acid oligomers to the conductive surface occurs covalently or by chemisorption or physisorption.

101. (NEW) The modified conductive surface according to claim 92, wherein one of the phosphoric-acid, carboxylic-acid or amine groups or a sugar group of the nucleic acid oligomer backbone is attached, covalently or by chemisorption or physisorption, to the conductive surface.

102. (NEW) The modified conductive surface according to claim 101, wherein a sugar-hydroxyl group fo the nucleic acid oligomer backbone is attached, covalently or by chemisorption or physisorption, to the conductive surface.

103. (NEW) A modified conductive surface according to claim 92, wherein a thiol group, a hydroxyl group, a carboxylic-acid group, or an amine group of a modified base of the nucleic acid oligomer is attached, covalently or by chemisorption or physisorption, to the conductive surface.

104. (NEW) The modified conductive surface according to claim 101, wherein the modified nucleic acid oligomer is bound to the conductive surface via a group at the end of the nucleic acid oligomer backbone or via a group of a terminal, modified base.

105. (NEW) The modified conductive surface according to claim 92, wherein branched or linear molecular moieties of any composition and chain length are attached, covalently or by chemisorption or physisorption, to the conductive surface and the modified nucleic acid oligomers are covalently attached to these molecular moieties.

106. (NEW)) The modified conductive surface according to claim 105, wherein the shortest continuous link between the conductive surface and the nucleic acid oligomer is a branched or linear molecular moiety having a chain length of 1-20 atoms.

107. (NEW) The modified conductive surface according to claim 105, wherein the shortest continuous ink between the conductive surface and the nucleic acid oligomer is a branched or linear molecular moiety having a chain length of 1-12 atoms.

108. (NEW) The modified conductive surface according to claim 105, wherein the branched or linear molecular moiety is attached to a phosphoric-acid group, a carboxylic-acid group, an amine group, or a sugar group of the nucleic acid oligomer backbone or a thiol, hydroxyl, carboxylic-acid, or amine group of a modified base of the nucleic acid oligomer.

109. (NEW) The modified nucleic acid oligomer according to claim 108, wherein the branched or linear molecular moiety is attached of a sugar-hydroxyl group fo the nucleic acid oligomer backbone.

110. (NEW) The modified conductive surface according to claim 108, wherein the branched or linear molecular moiety is bound to a phosphoric-acid, sugar-hydroxy, carboxylic-acid, or amine group at the end of the nucleic acid oligomer backbone or to a thiol, hydroxyl, carboxylic acid, or amine group of a terminal, modified base.

111. (NEW) The modified conductive surface according to claim 92, wherein predominantly one type of modified nucleic acid oligomer each is attached in a spatially delimited area of the conductive surface.

112. (NEW) The modified conductive surface according to claim 111, wherein only one type of modified nucleic acid oligomer each is attached in a spatially delimited area of the conductive surface.

113. (NEW) The method of producing a modified conductive surface according to claim 92, wherein at least one type of modified nucleic acid oligomer is applied to a conductive surface.

114. (NEW) The method of producing a modified conductive surface according to claim 112, wherein at least one type of nucleic acid oligomer is applied to a conductive surface and, subsequently, a modification of the nucleic acid oligomers is carried out.

115. (NEW) The method of producing a modified conductive surface according to claim 113, wherein the nucleic acid oligomers or the modified nucleic acid oligomers are hybridized with the respective complementary nucleic acid oligomer strand and applied to the conductive surface in the form of the double-strand hybrid.

116. (NEW) The method of producing a modified conductive surface according to claim 113, wherein the nucleic acid oligomer or the modified nucleic acid oligomer is applied to the conductive surface in the presence of further chemical compounds that are likewise attached to the conductive surface.

117. (NEW) The method of electrochemically detecting oligomer hybridization events, wherein at least one modified conductive surface according to claim 92, is brought into contact with nucleic acid oligomers and, subsequently, detection of the electrical communication between the redox-active moiety and the conductive surface takes place.

118. (NEW) The method according to claim 117, wherein detection takes place by cyclic voltammetry, amperometry, or conductivity measurement.

119. (NEW) The method according to claim 117, wherein electrochemical detection is initiated by photoinduced charged separation in the photoinducibly redox-active moiety attached to the conductive surface via a nucleic acid oligomer.

120. (NEW) The method according to claim 119, wherein the light irradiation for photoinduced charge separation in the photoinducibly redox-active moiety attached to the conductive surface via a nucleic acid oligomer is limited to an area of the conductive surface having at least one type of modified nucleic acid oligomer.

121. (NEW) The method according to claim 119, wherein the photoinducibly redox-active moiety's oxidized electron-donor molecule resulting from irradiation with light of a specific or any given wavelength is rereduced by a suitable free redox-active substance that is not bound to but in contact with the nucleic acid oligomer, i.e. it is restored to the state it was originally in prior to light irradiation.

122. (NEW) The method according to claim 119, wherein the photoinducibly redox-active moiety's reduced electron-acceptor molecule resulting form irradiation with light of a specific or any given wavelength is reoxidized by a suitable free redox-active substance that is not bound to but in contact with the nucleic acid oligomer, i.e. it is restored to the state it was originally in prior to light irradiation.

123. (NEW) The method of electrochemical detection according to claim 117, wherein the electrochemical detection is facilitated by a free redox-active substance that effectuates a thermal charge transfer to the redox-active moiety.

124. (NEW) The method according to claim 122, wherein the free redox-active substance that is not bound to but in contact with the nucleic acid oligomer is selectively oxidizable and reducible at a potential φ, where φ satisfies the condition $2.0\ V \geq \varphi \geq -2.0\ V$, measured against normal hydrogen electrode.

125. (NEW) The method according to claim 121, wherein the free redox-active substance that is not bound to but in contact with the nucleic acid oligomer is a free quinone, a free hexacyanoferrate(II) complex, a free sodium ascorbate, a free Ru(II)hexamine complex, or a free redox-active protein

126. (NEW) The method according to claim 125, wherein the free redox-active substance that is not bound to but in contact with the nucleic acid oligomer is a free cytochrome.

106. (NEW) The method according to claim 105, wherein the light irradiation for photoinduced charge separation in the photoinducibly redox-active moiety attached to the conductive surface via a nucleic acid oligomer is limited to an area of the conductive surface having one or more modified nucleic acid oligomer types.

107. (NEW) The method according to claim 105, wherein the photoinducibly redox-active moiety's oxidized electron-donor molecule or reduced electron-acceptor molecule resulting from irradiation with light of a specific or any given wavelength is rereduced or reoxidized by a suitable free redox-active substance not bound to, but in contact with the nucleic acid oligomer, i.e. the oxidized electron-donor molecule or reduced electron-acceptor molecule is restored to the state it was originally in prior to light irradiation.

108. (NEW) The method of electrochemical detection according to claim 103, wherein the electrochemical detection is facilitated by a free redox-active substance that effectuates a chemically induced charge transfer to the redox-active moiety.

109. (NEW) The method according to claim 107, wherein the free redox-active substance not bound to but in contact with the nucleic acid oligomer is selectively oxidizable and reducible at a potential φ, where φ satisfies the condition $2.0\ V \geq \varphi \geq -2.0\ V$, measured against normal hydrogen electrode.

110. (NEW) The method according to claim 107, wherein the free redox-active substance not bound to but in contact with the nucleic acid oligomer is a free quinone, a free hexacyanoferrate(II) complex, a free sodium ascorbate, a free Ru(II)hexamine complex, or a free redox-active protein, especially a free cytochrome.

REMARKS

Please enter the above before consideration of this application. With respect to the above newly entered claims, the subject matter of the claims is editorially revised and rewritten to bring that subject matter into conformity with the United States claim format.

In the event that there are any fee deficiencies or additional fees are payable, please charge the same or credit any overpayment to our Deposit Account (Account No. 04-0213).

Respectfully submitted,

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[012] Thus, although quantitative and extremely sensitive methods for DNA/RNA sequencing exist, these methods are time consuming, require painstaking sample preparation and expensive equipment, and are generally not available as portable systems.

[013] **DETAILED** DESCRIPTION OF THE INVENTION

[014] Therefore, it is the object of the present invention to create for detecting nucleic acid oligomer hybrids an apparatus and a method that do not exhibit the disadvantages of the background art.

[015] According to the present invention, this object is solved by the modified nucleic acid oligomer ~~according to independent claim 1~~, the method of producing a modified nucleic acid oligomer ~~according to independent claim 21~~, the modified conductive surface ~~according to independent claim 29~~, the method of producing a modified conductive surface ~~according to independent claim 44~~, and a method of electrochemically detecting nucleic acid oligomer hybridization events ~~according to independent claim 48~~.

[016] The following abbreviations and terms will be used in the context of the present invention:

[017]

Genetics

DNA	deoxyribonucleic acid
RNA	ribonucleic acid
PNA	peptide nucleic acid (synthetic DNA or RNA in which the sugar-phosphate moiety is replaced by an amino acid. If the sugar-phosphate moiety is replaced by the -NH-$(CH_2)_2$-N($COCH_2$-base)-CH_2CO- moiety, PNA will hybridize with DNA.)
A	adenine
G	guanine
C	cytosine

that, due to its being shaped as a cut-off cone that is hollow inside, coats a cyclophane or similar electron-donor/electron-acceptor complex.

[055] According to the present invention, a redox-active moiety is covalently bound to a nucleic acid oligomer by the reaction of the nucleic acid oligomer with the redox-active moiety or portions thereof (see also the section "~~Manner~~**Detailed Description** ~~of Executing~~ the Invention"). This bond can be achieved in four different ways:

[056] a) A free phosphoric-acid, sugar-C-3-hydroxy, carboxylic-acid, or amine group of the oligonucleotide backbone, especially a group at one of the two ends of the oligonucleotide backbone, is used as the reactive group for forming a bond at the nucleic acid oligomer. The free, terminal phosphoric-acid, sugar-C-3-hydroxy, carboxylic-acid, or amine groups exhibit increased reactivity and thus easily undergo typical reactions such as amidation with (primary or secondary) amino groups or with acid groups; esterification with (primary, secondary, or tertiary) alcohols or with acid groups; thioester formation with (primary, secondary, or tertiary) thioalcohols or with acid groups, or condensation of amine and aldehyde with subsequent reduction of the resultant CH=N bond to a CH_2-NH bond. The coupling group (acid, amine, alcohol, thioalcohol, or aldehyde function) required to covalently attach the redox-active moiety is either naturally present on the redox-active moiety or is obtained by chemically modifying the redox-active moiety. The attachment of the redox-active moiety may take place completely or in portions of the moiety with subsequent completion of the redox-active moiety (see below).

[057] b) The nucleic acid oligomer is modified with a reactive group at the oligonucleotide backbone or at a base via a covalently-attached molecular moiety (spacer) of any composition and chain length (longest continuous chain of atoms bound to one another), especially a chain length of 1 to 14. The modification preferably takes places at one of the ends of the oligonucleotide backbone or at a terminal base. An alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, or heteroalkynyl substituent, for example, may be used as the spacer. Possible simple reactions for forming the covalent bond between the redox-active moiety and the nucleic acid oligomer thus modified are, as described under a), amidation from an acid and amino group, esterification

amine group can react directly with the unmodified surface, as described under b) in this section. In addition, a further reactive group may be bound to the oligonucleotides near their second end, this reactive group, in turn, being attached, as described above, directly or via a spacer of any composition and chain length, especially a chain length of 1 - 14. Furthermore, as an alternative to this further reactive group, the redox-active moiety (completely or portions thereof) may be attached to this second end of the nucleic acid oligomer.

[072] Binding the nucleic acid oligomer to the conductive surface may take place before or after the redox-active moiety is attached to the nucleic acid oligomer. In the case of a redox-active protein/enzyme comprising apoprotein and cofactor(s), instead of the complete redox-active moiety, it is also possible for only the apoprotein, the apoprotein and a portion of the cofactors, or one or more of the cofactors to be attached and the redox-active moiety will be completed by subsequent reconstitution with the remaining missing portions. If a linked (at least bimolecular) electron-donor/electron-acceptor complex is used as the redox-active moiety, the electron acceptor (or donor) may, as described under b) or c) in the section "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," be attached to a terminal base, or in place of a terminal base, to the nucleic acid oligomer, and the electron donor (or acceptor) may be attached by subsequent covalent attachment to a reactive group of the electron acceptor (or donor) or, as described under a) in the section "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," by subsequent attachment to a terminal reactive group of the nucleic acid oligomer backbone at the same end (see also the section "~~Manner~~**Detailed Description** of ~~Executing~~ the Invention"). Alternatively, binding the nucleic acid oligomer to the conductive surface may take place before or after the spacer having a reactive group for binding the redox-active moiety is attached. Binding the already modified nucleic acid oligomer to the conductive surface, i.e. binding to the surface after the redox-active moiety is attached to the nucleic acid oligomer or after portions of the redox-active moiety are attached, or after the spacer having a reactive group for binding the redox-active moiety is attached, likewise takes place as described under a) to c) in this section.

strand oligonucleotide is used, the oligonucleotide double-strand is thermally dehybridized after the double-strand oligonucleotide is attached to the surface.

[077] Regarding the individual steps in "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," as well as in "Binding an Oligonucleotide to the Conductive Surface," it should be noted that, in the section "~~Manner~~**Detailed Description** of ~~Executing~~ the Invention," the various combination possibilities of the individual steps that lead to the same end result are demonstrated in an example (Figure 2).

[078] Method of Electrochemically Detecting Nucleic Acid Oligomer Hybrids

[079] Advantageously, according to the method of electrochemically detecting nucleic acid oligomer hybrids, multiple probe nucleic acid oligomers varying in sequence, ideally all necessary combinations of the nucleic acid oligomer, are applied to an oligomer (DNA) chip to detect the sequence of any target nucleic acid oligomer or (fragmented) target DNA, or in order to seek and sequence-specifically detect mutations in the target. For this purpose, the surface atoms or molecules of a defined area (a test site) on a conductive surface are linked with DNA/RNA/PNA nucleic acid oligomers having a known but arbitrary sequence, as described above. In a most general embodiment, however, the DNA chip may also be derivatized with a single probe oligonucleotide. Preferred probe nucleic acid oligomers are nucleic acid oligomers (e.g. DNA, RNA, or PNA fragments) of base length 3 to 50, preferably of length 5 to 30, particularly preferably of length 8 to 25. According to the present invention, a redox-active moiety is or becomes bound to the probe nucleic acid oligomers, as described below.

[080] The modification of the probe nucleic acid oligomers with a redox-active moiety may take place completely or in components of the redox-active moiety, either before or after the oligonucleotide probe is bound to the conductive surface. The various combination possibilities of the individual steps (reaction sequences) are demonstrated in the section "~~Manner~~**Detailed Description** of ~~Executing~~ the Invention" with the aid of Figure 2 using the example of a redox-active moiety bound to an electrode via a probe oligonucleotide.

potential at which the direct reaction of the free redox-active substance with the electrode can be significantly suppressed, and primarily electron transfers between the redox-active moiety and the electrode can be detected.

[090] If the redox-active moiety is a photoinducibly redox-active moiety, the redox activity of the moiety is triggered only by light of a specific or any given wavelength. According to the present invention, this property is used to advantage in that electrochemical detection is triggered only by radiating light onto the surface hybrid having the general structure elec-spacer-ds-oligo-spacer-moiety (surface hybrid with hybridized target) and is maintained, at most, as long as light irradiation continues. Thus, particularly in the case of amperometric detection, if a photoinducibly redox-active moiety is used, under certain external conditions, (rather long-lasting) current will flow only if light is radiated onto the surface hybrid. Such external conditions are for example the presence of a reducing agent (or oxidizing agent) suitable for reducing (or oxidizing) a photoinductively-formed oxidized donor D^+ (or reduced acceptor A^-) of the photoinducibly redox-active moiety, and applying to the electrode a potential at which a photoinductively-formed reduced acceptor A^- (or oxidized donor D^+) of the photoinducibly redox-active moiety can be oxidized (or reduced), but the non-reduced acceptor A (or the non-oxidized donor D) cannot be oxidized (or reduced). In the section "~~Manner~~**Detailed Description** of ~~Executing~~ the Invention," this is explained in greater detail using various examples of an elec-spacer-ss-oligo-spacer-moiety having a photoinducibly redox-active moiety. In this way, detection using a photoinducibly redox-active moiety can be spatially limited to a certain test site or group of test sites of the oligomer chip by restricting the light to this test site or group of test sites. According to the present invention, various test sites (nucleic acid oligomer combinations) of an oligomer chip can thus be applied to a shared, continuous, electrically-conductive surface. A particular test site or group of test sites can be addressed and amperometrically detected simply by applying a suitable external potential to the (entire) surface if precisely this test site or group of test sites is irradiated with light. The various test sites thus need not be applied to individual (micro-) electrodes that are electrically isolated from one another and individually controllable for applying a potential and reading out the current. Moreover, if surface hybrids having the general structure elec-spacer-ss-oligo-

[098] Fig. 6 Shows a detailed schematic diagram of the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-Q-ZnBChl of Figure 5 having gold as the solid support material, mercaptoethanol as the spacer (-S-CH_2CH_2- spacer) between the electrode and the oligonucleotide, and -CH_2-CH=CH-CO-NH-CH_2-CH_2-NH- as the spacer between the electron acceptor PQQ and the oligonucleotide, as well as a diagram of the sequence of the photoinduced electron transfer steps. The 12-bp probe oligonucleotide of the exemplary sequence 5'-TAGTCGGAAGCA-3' in the hybridized state is shown in detail.

[099] ~~**MANNER**~~**DETAILED DESCRIPTION** OF ~~EXECUTING~~ THE INVENTION

[100] A formation unit of an exemplary test site with hybridized target, Au-S$(CH_2)_2$-ds-oligo-spacer-UQ(RC) having the general structure elec-spacer-ds-oligo-spacer-moiety, is illustrated in Figure 4. In the context of the present invention, "formation unit" is understood to mean the smallest repeating unit of a test site. In the example in Figure 4, the surface is a gold electrode. The link between the gold electrode and the probe oligonucleotide was formed with the linker (HO-$(CH_2)_2$-S)$_2$, which was esterified with the terminal phosphate group at the 3'-end to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH and, following homolytic cleavage of the S-S bond at the gold surface, produced one Au-S bond each, with which 2-hydroxy-mercaptoethanol and mercaptoethanol-bridged oligonucleotide was coadsorbed on the surface. The photoinducibly redox-active moiety in the example in Figure 4 is the reaction center (RC) of the photosynthesizing bacteria *Rhodobacter sphaeroides*, a photoinducibly redox-active protein consisting of apoprotein and cofactors. In the application example, the RC, via its cofactor ubiquinone-50 (UQ) in what is known as the Q_A binding pocket of the RC, is covalently joined with the oligonucleotide, where free UQ was first provided with a reactive carboxylic-acid group (see Example 1), then covalently attached to the probe oligonucleotide via this carboxylic-acid group (amidation and dehydration of the terminal amino function of the -CH=CH-CO-NH-CH_2-CH_2-NH_2 linker attached at the C-5 position of the 5' thymine),

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(54) Title: METHOD FOR ELECTROCHEMICALLY DETECTING NUCLEIC ACID–OLIGOMER HYBRIDISATION EVENTS

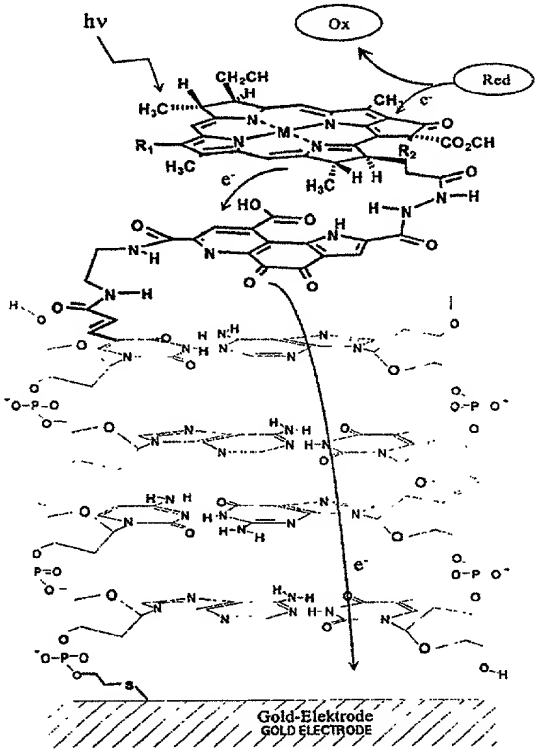
(54) Bezeichnung: VERFAHREN ZUR ELEKTROCHEMISCHEN DETEKTION VON NUKLEINSÄURE –OLIGOMER– HYBRIDISIERUNGSEREIGNISSEN

(57) Abstract

The invention relates to a method for electrochemically detecting sequence–specific nucleic acid–oligomer hybridisation events. DNA/RNA/PNA oligomer single strands which are bound to a conductive surface at one end and linked to a redoxactive unit at the other, free end, serve as a hybridisation matrix (probe). A proportion of the single strand oligonucleotides are hybridised by treatment with the oligonucleotide solution (target) being tested, with the result that the electrical communication between the conductive surface and the redoxactive unit, which is initially non– or barely existent, is increased. This enables a hybridisation event to be detected using electrochemical methods such as voltammetry, amperometry or conductance measurement.

(57) Zusammenfassung

Die vorliegende Erfindung betrifft ein Verfahren zur elektrochemischen Detektion von sequenzspezifischen Nukleinsäure–Oligomer–Hybridisierungsereignissen. Dabei dienen DNA–/RNA–/PNA–Oligomer–Einzelstränge, die mit einem Ende an einer leitfähigen Oberfläche gebunden und am anderen, freien Ende mit einer redoxaktiven Einheit verknüpft sind, als Hybridisierungsmatrix (Sonde). Durch Behandlung mit der zu untersuchenden Oligonukleotid–Lösung (Target) wird ein Teil der Einzelstrang–Oligonukleotide hybridisiert, wodurch die ursprünglich nicht oder nur schwach vorhandene elektrische Kommunikation zwischen der leitfähigen Oberfläche und der redoxaktiven Einheit erhöht wird. Somit wird die Detektion eines Hybridisierungsereignisses durch elektrochemische Verfahren wie Voltametrie, Amperometrie oder Leitfähigkeitsmessung ermöglicht.



[001] METHOD OF ELECTROCHEMICALLY DETECTING NUCLEIC ACID OLIGOMER HYBRIDIZATION EVENTS

[002] FIELD OF THE INVENTION

[003] The present invention is directed to a modified nucleic acid oligomer, as well as a method of electrochemically detecting sequence-specific nucleic acid oligomer hybridization events.

[004] BACKGROUND OF THE INVENTION

[005] Generally, gel-electrophoretic methods with autoradiographical or optical detection are used for DNA and RNA sequence analysis, for example in disease diagnosis, toxicological test procedures, genetic research and development, and the agricultural and pharmaceutical sectors.

[006] In the most significant gel-electrophoretic method with optical detection, the Sanger method, a solution containing DNA is divided into four samples. To differentiate the four samples, the primer (complementary starting sequence for replication) of each sample is covalently modified with a fluorescent dye that emits at a distinct wavelength. Starting at the primer, each sample is enzymatically replicated by DNA polymerase I. In addition to the requisite deoxyribonucleoside triphosphates of the bases A (adenine), T (thymine), C (cytosine), and G (guanine), each reaction mixture also contains sufficient 2',3'-dideoxy analog of one of these nucleoside triphosphates as a blocking base (one of each of the four possible blocking bases per sample) in order to terminate replication at all possible binding sites. After the four samples are combined, all lengths of replicated DNA fragments having blocking-base-specific fluorescence result and can be sorted gel-electrophoretically by length and characterized by fluorescent spectroscopy.

[007] Another optical detection method is based on the attachment of fluorescent dyes such as ethidium bromide on oligonucleotides. In comparison with a free solution of the dye, the fluorescence of such dyes changes

drastically upon association with double-stranded DNA or RNA and can therefore be used to detect hybridized DNA or RNA.

[008] In radio labeling, ^{32}P is built into the phosphate skeleton of the oligonucleotides, ^{32}P usually being added to the 5'-hydroxyl end by polynucleotide kinase. Thereafter, the labeled DNA is preferably cleaved, under defined conditions, at one of each of the four nucleotide types, such that an average of one cleavage per chain results. Thus, for a given base type, there are present in the reaction mixture chains extending from the ^{32}P-label to the position of that base (if there are multiple appearances of the base, chains of varying lengths will result accordingly). The four fragment mixtures are then gel-electrophoretically separated on four lanes. Thereafter, an autoradiogram of the gel is prepared, from which the sequence can be read directly.

[009] Some years ago, a further method of DNA sequencing was developed on the basis of optical (or autoradiographical) detection, namely sequencing by oligomer hybridization (cf. e.g. Drmanac et al., Genomics 4, (1989), pp. 114-128 or Bains et al., Theor. Biol. 135, (1988), pp. 303-307). In this method, a complete set of short oligonucleotides or nucleic acid oligomers (probe oligonucleotides), e.g. all 65,536 possible combinations of bases A, T, C, and G of an oligonucleotide octamer, are bound to a support material. The attachment occurs in an ordered grid comprising 65,536 test sites, each rather large amount of an oligonucleotide combination defining one test site, and the position of each individual test site (oligonucleotide combination) being known. On such a hybridization matrix, the oligomer chip, a DNA fragment whose sequence is to be determined (the target) is labeled with fluorescent dye (or ^{32}P) and hybridized under conditions that allow only one specific double-strand formation. In this way, the target DNA fragment binds only to those nucleic acid oligomers (in this example to the octamers) whose complementary sequence corresponds exactly to a portion (an octamer) of its own sequence. Thus, all of the nucleic acid oligomer sequences (octamer sequences) present in the fragment are determined by optical (or autoradiographical) detection of

the binding position of the hybridized DNA fragment. Due to the overlapping of neighboring nucleic acid oligomer sequences, the sequential sequence of the DNA fragment can be determined using appropriate mathematical algorithms. One of the advantages of this method lies in the miniaturization of the sequencing and thus in the enormous amount of data that can be simultaneously captured in one operation. In addition, primer and gel-electrophoretic separation of the DNA fragments can be dispensed with. This principle is exemplified in Figure 1 for a 13-base-long DNA fragment.

[010] The use of radioactive labels in DNA/RNA sequencing is associated with several disadvantages, such as elaborate, legally required safety precautions in dealing with radioactive materials, radiation exposure, limited spatial resolution capacity (maximum 1 mm^2), and sensitivity that is high only when the radiation of the radioactive fragments act on an X-ray film for an appropriately long time (hours to days). Although the spatial resolution can be increased by additional hardware and software, and the detection time can be decreased by using beta scanners, both of these involve considerable additional costs.

[011] Some of the fluorescent dyes that are commonly used to label the DNA (e.g. ethidium bromide) are mutagenic and require appropriate safety precautions, as does the use of autoradiography. In nearly every case, the use of optical detection requires the use of one or more laser systems, and thus experienced personnel and appropriate safety precautions. The actual detection of the fluorescence requires additional hardware, such as optical components for amplification and, in the case of varying excitation and query wavelengths as in the Sanger method, a control system. Thus, depending on the required excitation wavelengths and the desired detection performance, considerable investment costs may result. In sequencing by hybridization on an oligomer chip, detection is even more costly since, in addition to the excitation system, high-resolution CCD cameras (charge coupled device cameras) are needed for the two-dimensional detection of fluorescent spots.

[012] Thus, although quantitative and extremely sensitive methods for DNA/RNA sequencing exist, these methods are time consuming, require painstaking sample preparation and expensive equipment, and are generally not available as portable systems.

[013] DESCRIPTION OF THE INVENTION

[014] Therefore, it is the object of the present invention to create for detecting nucleic acid oligomer hybrids an apparatus and a method that do not exhibit the disadvantages of the background art.

[015] According to the present invention, this object is solved by the modified nucleic acid oligomer according to independent claim 1, the method of producing a modified nucleic acid oligomer according to independent claim 21, the modified conductive surface according to independent claim 29, the method of producing a modified conductive surface according to independent claim 44, and a method of electrochemically detecting nucleic acid oligomer hybridization events according to independent claim 48.

[016] The following abbreviations and terms will be used in the context of the present invention:

[017]

Genetics

DNA	deoxyribonucleic acid
RNA	ribonucleic acid
PNA	peptide nucleic acid (synthetic DNA or RNA in which the sugar-phosphate moiety is replaced by an amino acid. If the sugar-phosphate moiety is replaced by the -NH-$(CH_2)_2$-N($COCH_2$-base)-CH_2CO- moiety, PNA will hybridize with DNA.)
A	adenine
G	guanine
C	cytosine

T	thymine
U	uracil
base	A, G, T, C, or U
bp	base pair
nucleic acid	At least two covalently-joined nucleotides or at least two covalently-joined pyrimidine (e.g. cytosine, thymine, or uracil) or purine bases (e.g. adenine or guanine). The term nucleic acid refers to any backbone of the covalently-linked pyrimidine or purine bases, such as the sugar-phosphate backbone of DNA, cDNA, or RNA, a peptide backbone of PNA, or analogous structures (e.g. a phosphoramide, thiophosphate, or dithiophosphate backbone). The essential feature of a nucleic acid according to the present invention is that it can sequence-specifically bind naturally occurring cDNA or RNA.
nucleic acid oligomer	Nucleic acid of a base length that is not further specified (e.g. nucleic acid octamer: a nucleic acid having any backbone in which 8 pyrimidine or purine bases are covalently bound to one another).
oligomer	Equivalent to nucleic acid oligomer.
oligonucleotide	Equivalent to oligomer or nucleic acid oligomer, e.g. a DNA, PNA, or RNA fragment of a base length that is not further specified.
oligo	Abbreviation for oligonucleotide.
primer	Initial complementary fragment of an oligonucleotide, the base length of the primer being only approx. 4-8 bases. Serves as the starting point for enzymatic replication of the oligonucleotide.
mismatch	To form the Watson-Crick double-stranded oligonucleotide structure, the two single-strands hybridize in such a way that the A (or C) base of one strand forms hydrogen bonds with the

	T (or G) base of the other strand (in RNA, T is replaced by uracil). Any other base pairing does not form hydrogen bonds, distorts the structure and is referred to as a "mismatch."
ss	single-strand
ds	double-strand

[018]

Photoinducibly and Chemically-Inducibly Redox-Active Moieties

redox-active moiety	A photoinducibly redox-active moiety or a chemically-inducibly redox-active moiety
electron donor	In the context of the present invention, the term "electron donor" refers to a component of a photoinducibly or chemically-inducibly redox-active moiety. An electron donor is a molecule that can transfer an electron to an electron acceptor, directly or under the influence of certain external conditions. One such external condition is light absorption by the electron donor or acceptor of a photoinducibly redox-active moiety. Upon irradiation with light of a specific or any given wavelength, the electron donor "D" gives up an electron to the/an electron acceptor "A" and a charge-separated state D^+A^- is formed, at least temporarily, comprising the oxidized donor and the reduced acceptor. Another such external condition may be the oxidation or reduction of the electron donor or acceptor of the chemically-inducibly redox-active moiety by an external oxidizing or reducing agent, so for example the transfer of an electron to the electron donor by a reducing agent, or the release of an electron by the electron acceptor to an oxidizing agent. These oxidizing or reducing agents may be external redox-active substances, i.e. they are not covalently joined with but in contact with the redox-active moiety, the nucleic acid oligomer, or the conductive surface, for example via the solution added to the modified conductive surface, or they

are covalently joined with the nucleic acid oligomer, the oxidizing or reducing agent being covalently attached to the nucleic acid oligomer at a site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the redox-active moiety, preferably at the oligonucleotide end opposite the modification with the redox-active moiety, near the conductive surface. The ability to act as an electron donor or acceptor is relative, i.e. a molecule that acts as an electron donor toward another molecule, directly or under the influence of certain external conditions, may also act as an electron acceptor toward that molecule under differing experimental conditions, or toward a third molecule under the same or differing experimental conditions.

electron acceptor

In the context of the present invention, the term "electron acceptor" refers to a component of a photoinducibly or chemically-inducibly redox-active moiety. An electron acceptor is a molecule that can take up an electron from an electron donor, directly or under the influence of certain external conditions. One such external condition is light absorption by the electron donor or acceptor of a photoinducibly redox-active moiety. Upon irradiation with light of a specific or any given wavelength, the electron donor "D" gives up an electron to the/an electron acceptor "A" and a charge-separated state D^+A^- is formed, at least temporarily, comprising the oxidized donor and reduced acceptor. Another such external condition may be the oxidation or reduction of the electron donor or acceptor of the chemically-inducibly redox-active moiety by an external oxidizing or reducing agent, so for example the transfer of an electron to the electron donor by a reducing agent, or the release of an electron by the

electron acceptor to an oxidizing agent. These oxidizing or reducing agents may be external redox-active substances, i.e. they are not covalently joined with but in contact with the redox-active moiety, the nucleic acid oligomer, or the conductive surface, for example via the solution added to the modified conductive surface, or they are covalently joined with the nucleic acid oligomer, the oxidizing or reducing agent being covalently attached to the nucleic acid oligomer at a site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the redox-active moiety, preferably at the oligonucleotide end opposite the modification with the redox-active moiety, near the conductive surface. The ability to act as an electron acceptor or donor is relative, i.e. a molecule that acts as an electron acceptor toward another molecule, directly or under the influence of certain external conditions, may also act as an electron donor toward that molecule under differing experimental conditions, or toward a third molecule under the same or differing experimental conditions.

electron-donor molecule — Equivalent to an electron donor.

electron-acceptor molecule — Equivalent to an electron acceptor.

oxidizing agent — A chemical compound (chemical substance) that oxidizes another chemical compound (chemical substance, electron donor, electron acceptor) by taking up electrons from this other chemical compound (chemical substance, electron donor, electron acceptor). An oxidizing agent behaves analogously to an electron acceptor, but is used in the context of the present invention to denote an external electron acceptor not directly belonging to the

	photoinducibly or chemically-inducibly redox-active moiety. In this context, "not directly" means that the oxidizing agent is either a free redox-active substance that is not bound to but in contact with the nucleic acid oligomer, or that the oxidizing agent is covalently attached to the nucleic acid oligomer, but at a nucleic acid oligomer site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the (photoinducibly) redox-active moiety. In particular, the electrode may represent the oxidizing agent.
reducing agent	A chemical compound (chemical substance) that reduces another chemical compound (chemical substance, electron donor, electron acceptor) by giving up electrons to this other chemical compound (chemical substance, electron donor, electron acceptor). A reducing agent behaves analogously to an electron donor but is used in the context of the present invention to denote an external electron donor not directly belonging to the photoinducibly or chemically-inducibly redox-active moiety. In this context, "not directly" means that the reducing agent is either a free redox-active substance that is not bound to but in contact with the nucleic acid oligomer, or that the reducing agent is covalently attached to the nucleic acid oligomer, but at a nucleic acid oligomer site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the redox-active moiety. In particular, the electrode may represent the reducing agent.
photoinducible	Photoinducible means that a certain property is exhibited only upon irradiation with light of a specific or any given wavelength. For example, a photoinducibly redox-active moiety exhibits its redox activity, in other words its property of

	carrying out a charge separation within the photoinducibly redox-active moiety under certain external conditions, for example forming the state D^+A^- and giving up electrons to another suitable oxidizing agent or taking up electrons from another suitable reducing agent, only upon irradiation with light of a specific or any given wavelength. A further example is the photoinducibly reactive group, i.e. a group that becomes reactive only upon irradiation with light of a specific or any given wavelength.
redox-active	Redox-active refers to the property of a redox-active moiety of giving up electrons to a suitable oxidizing agent or taking up electrons from a suitable reducing agent under certain external conditions, or the property of a redox-active substance of giving up electrons to a suitable electron acceptor or taking up electrons from a suitable electron donor under certain external conditions.
free redox-active substance	A free oxidizing or reducing agent not covalently joined with but in contact with the redox-active moiety, the nucleic acid oligomer, or the conductive surface, for example via the solution added to the modified conductive surface, the free redox-active substance being able to be for example an uncharged molecule, any salt, or a redox-active protein or enzyme (oxido-reductase). The free redox-active substance is characterized in that it can rereduce the oxidized donor (or reoxidize the reduced acceptor) of the photoinducibly redox-active moiety, or in that the free redox-active substance can reduce the donor (or oxidize the acceptor) of the chemically-inducibly redox-active moiety.
photoinducibly redox-active moiety	Generic term for a moiety that includes one or more electron-donor molecules and one or more electron-acceptor

molecules, this (these) electron-donor molecule(s) and/or electron-acceptor molecule(s) being able to be embedded in one or more macromolecules. Electron donor(s) and electron acceptor(s) may be joined with one another via one or more covalent or ionic bonds, via hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance, covalent links being able to be direct or indirect links (e.g. via a spacer, but not via a nucleic acid oligomer). In addition, electron donor(s) and/or electron acceptor(s) that are embedded in one or more macromolecule(s) may be joined with the macromolecule(s) via covalent attachment to the macromolecule(s), via encapsulation in suitable molecular cavities (binding pockets) of the macromolecule(s), via ionic bonds, hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance between the macromolecule(s) and the electron-donor molecule(s) and/or electron-acceptor molecule(s). If the photoinducibly redox-active moiety is composed of multiple macromolecules, the binding of the macromolecules to one another may likewise take place covalently, ionically, via hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance. In addition to comprising electron donor(s) and electron acceptor(s) or electron donor(s), electron acceptor(s), and macromolecule(s), essential features of the photoinducibly redox-active moiety are: (i) in the forms relevant to the present invention (electron donor(s) and electron acceptor(s) in their original state or in the oxidized or reduced state), the moiety is stable and does not dissociate into its components, (ii) the moiety includes no

nucleic acid, (iii) the moiety's composition comprising electron donor(s) and electron acceptor(s) or electron donor(s), electron acceptor(s), and macromolecule(s) can be recognized by a person skilled in the art, regardless of the bond between the components, since, in principle, they may also occur as single molecules, and (iv) under the same or similar external conditions, electron donor(s) and electron acceptor(s) of the photoinducibly redox-active moiety in the form of single molecules in solution act as electron donor(s) and electron acceptor(s), i.e. even in the case of free dissolved electron donor(s) and electron acceptor(s), an electron can be transferred from the dissolved electron donor(s) to the dissolved electron acceptor(s), directly or under the influence of certain external conditions, depending on the conditions that lead to an electron transfer within the photoinducibly redox-active moiety. The photoinducibly redox-active moiety may be for example any photoinducibly redox-active protein/enzyme or any photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex. Upon irradiation with light of a specific or any given wavelength, the/an electron donor gives up an electron to one of the electron acceptors and a charge-separated state D^+A^- is formed, at least temporarily, comprising an oxidized donor and a reduced acceptor. This process within the photoinducibly redox-active moiety is referred to as photoinduced charge separation. Given appropriately chosen external conditions, the photoinducibly redox-active moiety exhibits its redox activity, in other words its property of giving up electrons to a suitable oxidizing agent or taking up electrons from a suitable reducing agent, only in a charge-separated state, since the reducing

agent transfers (or the oxidizing agent takes up) electrons only to the oxidized donor (or from the reduced acceptor) of the photoinducibly redox-active moiety, for example in the presence of a reducing agent that can reduce D^+ but not D (or in the presence of an oxidizing agent that can oxidize A^- but not A). In particular, this oxidizing or reducing agent may also be an electrode, the photoinducibly redox-active moiety being able to give up an electron to an electrode (or take up an electron therefrom) only subsequent to photoinduced charge separation, for example if the electrode is set to a potential at which A^- but not A is oxidized (or at which D^+ but not D is reduced).

chemically-inducibly redox-active moiety

Corresponds to a photoinducibly redox-active moiety in composition and manner of functioning, but in contrast to the manner in which a photoinducibly redox-active moiety functions, photoactivation is precluded as an external condition for developing the redox activity of the redox-active moiety. The redox-active moiety may be for example any redox-active protein/enzyme or any redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex. Given appropriately chosen external conditions, the redox-active moiety exhibits its redox activity, for example its property of giving up electrons to a suitable oxidizing agent, only after an electron is transferred from a reducing agent to the/an electron donor "D" that can transfer an electron to the acceptor "A" only in the reduced state "D^-," and the oxidizing agent takes up electrons only from this reduced acceptor "A^-" of the redox-active moiety, in other words in the presence of an oxidizing agent that can oxidize A^- but not A (successive charge transfer). In particular, this oxidizing agent may also be an

electrode, for example if the electrode is set to a potential at which A^- but not A is oxidized. Conversely, given differently chosen external conditions, the redox-active moiety can exhibit its redox activity, for example its property of taking up electrons from a suitable reducing agent, only after an electron is transferred from an electron acceptor "A" to an oxidizing agent, if only the oxidized acceptor "A^+" can take up an electron from the donor D and the reducing agent can transfer electrons only to the oxidized donor "D^+" of the redox-active moiety, for example in the presence of a reducing agent that can reduce D^+ but not D (successive charge transfer). In particular, this reducing agent may also be an electrode, for example if the electrode is set to a potential at which D^+ but not D is reduced.

photoinducibly redox-active protein/enzyme

Usually consists of what is known as 'apoprotein' – the preferred macromolecule(s) of the present invention – and cofactors – electron donor(s) and electron acceptor(s) within the meaning of the present invention. Photoinduced charge separation within the photoactivatable redox-active protein/enzyme is triggered by light of a specific or any given wavelength. Thus, for example, in the photosynthetic reaction center (RC), cofactors in the form of a primary electron donor P and multiple different electron acceptors A, including quinone cofactor(s) Q, are embedded in a protein matrix, thus forming a "polymolecular" moiety (cf. Structure 1). In this case, the embedding takes place via encapsulation of the cofactors in suitable cavities (known as binding pockets) of the protein matrix comprising multiple protein sub-units. In the case of some naturally occurring RCs, both the protein sub-units and the encapsulation of the cofactors in the protein matrix are

	realized via non-covalent bonds. Upon irradiation with light of a suitable wavelength, the primary donor gives up an electron to one of the electron acceptors and a charge-separated RC-state P^+A^-, and especially the state P^+Q^-, is formed, at least temporarily, comprising the initially neutral cofactors.
chemically-inducibly redox-active protein/ enzyme	Corresponds to a photoinducibly redox-active protein/enzyme in composition and manner of functioning, but in contrast to the manner in which a photoinducibly redox-active protein/enzyme functions, photoactivation is precluded as an external condition for developing the redox activity of the redox-active moiety; the chemically-inducibly redox-active protein/enzyme usually consists of what is known as apoprotein – the preferred macromolecule(s) of the present invention – and cofactors – electron donor(s) and electron acceptor(s) within the meaning of the present invention. The redox-active protein's/enzyme's property of successive charge transfer is triggered by a free redox-active substance (substrate).
photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex	A compound comprising one or more electron-donor molecules D1, D2, D3, etc. and at least one or more suitable electron-acceptor molecules A1, A2, A3, etc., the electron donor(s) and electron acceptor(s) being joined with one another via one or more covalent or ionic bonds, via hydrogen bonds, van der Waals bridges, via π-π-interaction, or via coordination by means of electron-pair donation and acceptance. Covalent links in this sense may be direct or indirect links (e.g. via a spacer, but not via a nucleic acid oligomer). In addition to comprising electron donor(s) and electron acceptor(s), essential features of the photoinducibly redox-active, linked, at least bimolecular electron-

donor/electron-acceptor complex are: (i) in the forms relevant to the present invention (electron donor(s) and electron acceptor(s) in their original state or in the oxidized or reduced state), the electron-donor/electron-acceptor complex is stable and does not dissociate into its components, (ii) the moiety includes no nucleic acid, (iii) the at least bimolecular electron-donor/electron-acceptor complex's composition comprising electron donor(s) and electron acceptor(s) can be recognized by a person skilled in the art, regardless of the bond between the components, and (iv) under the same or similar external conditions, electron donor(s) and electron acceptor(s) of the photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex, also in the form of single molecules in solution, act as electron donor(s) and electron acceptor(s), i.e. even in the case of free dissolved electron donor(s) and electron acceptor(s), an electron can be transferred from the dissolved electron donor(s) to the dissolved electron acceptor(s), directly or under the influence of certain external conditions, depending on the conditions that lead to an electron transfer within the photoinducibly redox-active moiety. In its manner of functioning that is relevant to the present invention, the photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex corresponds to a photoinducibly redox-active protein/enzyme, i.e. here, too, irradiation with light of a suitable wavelength leads to photoinduced charge separation and, at least temporarily, a charge-separated state D^+A^- is formed (wherein D stands for any D1, D2, D3, etc. and A for any A1, A2, A3, etc.). In the expression "photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex,"

	the term "at least bimolecular" indicates that the complex is composed of at least one electron donor and at least one electron acceptor, even if the donor is directly (or indirectly via a spacer) covalently joined with the acceptor.
chemically-inducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex	Corresponds to a photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex in composition and manner of functioning, but in contrast to the manner in which a photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex functions, photoactivation is precluded as an external condition for developing the redox activity of the redox-active moiety. Given appropriately chosen external conditions, the redox-active moiety exhibits its redox activity, for example its property of giving up electrons to a suitable oxidizing agent, only after an electron is transferred from a reducing agent to the/an electron donor "D" that can transfer an electron to the acceptor "A" only in the reduced state "D^-," and the oxidizing agent takes up electrons only from this reduced acceptor "A^-" of the redox-active moiety, in other words in the presence of an oxidizing agent that can oxidize A^- but not A (successive charge transfer). In particular, this oxidizing agent may also be an electrode, for example if the electrode is set to a potential at which A^- but not A is oxidized. Conversely, given differently chosen external conditions, the redox-active moiety can exhibit its redox activity, for example its property of taking up electrons from a suitable reducing agent, only after an electron is transferred from an electron acceptor "A" to an oxidizing agent, if only the oxidized acceptor "A^+" can take up an electron from the donor D and the reducing agent can transfer electrons only to the oxidized donor "D^+" of the redox-active

	moiety, for example in the presence of a reducing agent that can reduce D^+ but not D (successive charge transfer). In particular, this reducing agent may also be an electrode, for example if the electrode is set to a potential at which D^+ but not D is reduced. In the expression "redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex," the term "at least bimolecular" indicates that the complex is composed of at least one electron donor and at least one electron acceptor, even if the donor is directly (or indirectly via a spacer) covalently joined with the acceptor.
RC	Reaction center. An example of a photoinducibly redox-active protein/enzyme. The protein/enzyme is what is known as a pigment/protein complex composed of apoprotein comprising multiple protein sub-units and multiple cofactors (known as pigments in the example of RC). The first steps of light-driven charge separation in bacterial or vegetable photosynthesis take place in such pigment/protein complexes. For example, the RC of photosynthesizing bacteria of the strain *Rhodobacter sphaeroides* (cf. Structure 1) consists of three protein sub-units and eight cofactors (pigments). The cofactors, a bacteriochlorophyll dimer P, two bacteriochlorophyll monomers B_A and B_B, two bacteriopheophytin monomers H_A and H_B, and two ubiquinone-50 (UQ) molecules Q_A and Q_B, are localized in the respective protein binding pockets (i.e. the P, B_A, etc. binding pockets).
Q_A protein binding pocket	A protein binding pocket (or protein environment) in which the quinone cofactor Q_A is located. In the RC of *Rhodobacter sphaeroides*, for example, the quinone cofactor Q_A is a ubiquinone-50 (cf. Structure 1).

Q_A binding pocket	Q_A protein binding pocket

[019] Chemical Substances/Groups

ZnBChl	Zinc bacteriochlorophyll (Formula 11 with M = Zn)
Q	Generally quinone; in Example 3 and the passages referring thereto, Q is a modified anthraquinone or pyrrolloquinoline quinone (PQQ).
UQ	Ubiquinone-50, RC cofactor and temporary electron acceptor, for example in the RC of the photosynthesizing bacteria of *Rhodobacter sphaeroides* or *Rhodopseudomonas viridis*.
$(cyt\ c_2)^{2+}$	Reduced form of the cytochrome c_2, a freely movable hemoprotein that, in bacterial photosynthesis in *Rhodobacter sphaeroides,* reduces the oxidized primary donor P^+ to P; an example of a redox-active substance.
PQQ	Pyrroloquinoline quinone; corresponds to 4,5-dihydro-4,5-dioxo-1H-pyrrolo-[2,3-f]-quinoline-2,7,9-tricarboxylic acid
EDTA	ethylenediamine tetraacetate (sodium salt)
sulfo-NHS	N-hydroxysulfosuccinimide
EDC	(3-dimethylaminopropyl)-carbodiimide
HEPES	N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
Tris	trishydroxymethylamino methane
alkyl	The term "alkyl" refers to a saturated hydrocarbon group that is straight-chain or branched (e.g. ethyl, 2,5-dimethylhexyl, or isopropyl, etc.). When "alkyl" is used to indicate a linker or spacer, the term refers to a group having two available valences for covalent linkage (e.g. $-CH_2CH_2-$, $-CH_2C(CH_3)_2CH_2CH_2C(CH_3)_2CH_2-$ or $-CH_2CH_2CH_2-$, etc.). Alkyl groups preferred as substituents or side chains R are those having a chain length of 1 – 30 (the longest continuous chain of atoms

	covalently bound to one another). Alkyl groups preferred as linkers or spacers are those having a chain length of 1 – 20, especially a chain length of 1 – 14, the chain length here representing the shortest continuous link between the structures joined via the linker or spacer, in other words between the two molecules or between a surface atom, surface molecule, or surface molecule group and another molecule.
alkenyl	Alkyl groups in which one or more of the C-C single bonds are replaced by C=C double bonds.
alkynyl	Alkyl or alkenyl groups in which one or more of the C-C single or C=C double bonds are replaced by C≡C triple bonds.
heteroalkyl	Alkyl groups in which one or more of the C-H bonds or C-C single bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
heteroalkenyl	Alkenyl groups in which one or more C-H bonds, C-C single or C=C double bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
heteroalkynyl	Alkynyl groups in which one or more of the C-H bonds, C-C single, C=C double or C≡C triple bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
linker	A molecular link between two molecules or between a surface atom, surface molecule, or surface molecule group and another molecule. Linkers can usually be purchased in the form of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, or heteroalkynyl chains, the chain being derivatized in two places with (identical or different) reactive groups. These groups form a covalent chemical bond in simple/known chemical reactions with the appropriate reaction partners. The reactive groups may also be photoactivatable, i.e. the reactive groups are activated only by light of a specific or any given wavelength.

	Preferred linkers are those having a chain length of 1 - 20, especially a chain length of 1 - 14, the chain length here representing the shortest continuous link between the structures to be joined, in other words between the two molecules or between a surface atom, surface molecule, or surface molecule group and another molecule.
spacer	A linker that is covalently attached via the reactive groups to one or both of the structures to be joined (see linker). Preferred spacers are those having a chain length of 1 - 20, especially a chain length of 1 - 14, the chain length representing the shortest continuous link between the structures to be joined.
(n x HS-spacer)-oligo	A nucleic acid oligomer to which n thiol functions are each attached via a spacer, each spacer being able to have a different chain length (the shortest continuous link between the thiol function and the nucleic acid oligomer), especially any chain length between 1 and 14. These spacers, in turn, may be bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been affixed thereto by modification, and "n" is any integer, especially a number between 1 and 20.
(n x R-S-S-spacer)-oligo	A nucleic acid oligomer to which n disulfide functions are each attached via a spacer, the disulfide function being saturated by any residue R. Each spacer for attaching the disulfide function to the nucleic acid oligomer may have a different chain length (shortest continuous link between the disulfide function and the nucleic acid oligomer), especially any chain length between 1 and 14. These spacers, in turn, may be bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been affixed thereto by modification. The variable n is any integer, especially a number

	between 1 and 20.
oligo-spacer-S-S-spacer-oligo	Two identical or different nucleic acid oligomers that are joined with each other via a disulfide bridge, the disulfide bridge being attached to the nucleic acid oligomers via any two spacers, the two spacers being able to have differing chain lengths (the shortest continuous link between the disulfide bridge and the respective nucleic acid oligomer), especially any chain length between 1 and 14, and these spacers, in turn, being able to be bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been affixed thereto by modification.

[020] Modified Surfaces/Electrodes

mica	Muscovite lamina, a support material for the application of thin films.
Au-S-$(CH_2)_2$-ss-oligo-spacer-UQ(RC)	Gold film on mica having a covalently applied monolayer of derivatized 12-bp single-strand DNA oligonucleotide (sequence: TAGTCGGAAGCA). Here, the oligonucleotide's terminal phosphate group at the 3'-end is esterified with $(HO-(CH_2)_2-S)_2$ to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH, the S-S bond being homolytically cleaved and producing one Au-S-R bond each. The terminal thymine base at the 5'-end of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH_2-CH_2-NH_2, this residue, in turn, being joined via its free amino group with the carboxylic-acid group of the modified ubiquinone-50 by amidation. Thereafter, the UQ is reconstituted with the remaining RC.
Au-S-$(CH_2)_2$-ds-oligo-spacer-UQ(RC)	*Au-S-$(CH_2)_2$-ss-oligo-spacer-UQ(RC)* hybridized with the oligonucleotide that is complementary to the ss-oligo (sequence: TAGTCGGAAGCA).
Au-S-$(CH_2)_2$-ss-oligo-	Identical to *Au-S-$(CH_2)_2$-ss-oligo-spacer-UQ(RC)* with the

spacer-Q-ZnBChl	exception that, instead of the RC attached via UQ, Q-ZnBChl is attached as the photoinducibly redox-active moiety.
Au-S-$(CH_2)_2$-ds-oligo-spacer-Q-ZnBChl	*Au-S-$(CH_2)_2$-ss-oligo-spacer-Q-ZnBChl* hybridized with the oligonucleotide that is complementary to the ss-oligo (sequence: TAGTCGGAAGCA).

[021]

Electrochemistry

E	The electrode potential on the working electrode.
E_{Ox}	Potential at maximum current of the oxidation of a reversible electrooxidation or electroreduction.
i	current density (current per cm^2 of electrode surface)
cyclic voltammetry	Recording a current-voltage curve. Here, the potential of a stationary working electrode is changed linearly as a function of time, starting at a potential at which no electrooxidation or electroreduction occurs, up to a potential at which a species that is dissolved or adsorbed on the electrode is oxidized or reduced (i.e. a current flows). After running through the oxidation or reduction operation, which produces in the current-voltage curve an initially increasing current and, after reaching a maximum, a gradually decreasing current, the direction of the potential feed is reversed. The behavior of the products of electrooxidation or electroreduction is then recorded in a reverse run.
amperometry	Recording a current-time curve. Here, the potential of a stationary working electrode is set, for example by a potential jump, to a potential at which the electrooxidation or electroreduction of a dissolved or adsorbed species occurs, and the flowing current is recorded as a function of time.

[022] The present invention is directed to a nucleic acid oligomer that is modified by chemically binding a redox-active moiety. The redox-active moiety is either a photoinducibly redox-active moiety or a chemically-inducibly redox-active moiety. Subsequent to photoinduced release of an electron to an external oxidizing agent, for example an electrode, or taking up an electron

from an external reducing agent, for example an electrode, the photoinducibly redox-active moiety may be rereduced or reoxidized by a free redox-active substance, in other words it may be restored to its original state. After giving up an electron to an external oxidizing agent, the chemically induced redox-active moiety may be reduced by an external reducing agent, for example an electrode, or after taking up an electron from an external reducing agent, be oxidized by an external oxidizing agent, for example an electrode.

[023] In the context of the present invention, a compound comprising at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine (e.g. cytosine, thymine, or uracil) or purine bases (e.g. adenine or guanine), preferably a DNA, RNA, or PNA fragment, is used as the nucleic acid oligomer. In the present invention, the term "nucleic acid" refers to any backbone of the covalently-joined pyrimidine or purine bases, such as the sugar-phosphate backbone of DNA, cDNA, or RNA, a peptide backbone of PNA, or analogous backbone structures such as a thiophosphate, a dithiophosphate, or a phosphoramide backbone. An essential feature of a nucleic acid within the meaning of the present invention is that it can sequence-specifically bind naturally occurring cDNA or RNA. The terms "(probe) oligonucleotide," "nucleic acid," and "oligomer" are used as alternatives to the term "nucleic acid oligomer."

[024] In the context of the present invention, the term "electron acceptor" or "electron-acceptor molecule" and the term "electron donor" or "electron-donor molecule" refer to a component of a redox-active moiety.

[025] In the context of the present invention, a "redox-active moiety" is understood to be any moiety that includes one or more electron-donor molecules and one or more electron-acceptor molecules. The electron-donor molecule(s) or molecular moiety (moieties) and electron-acceptor molecule(s) or molecular moiety (moieties) of this redox-active moiety may be joined with one another via one or more covalent or ionic bonds, via hydrogen bonds, van der Waals bridges, via π-π-interaction, or via coordination by means of electron-pair donation and acceptance, the covalent bonds being able to be direct or indirect bonds (e.g. via a spacer, but not via a nucleic acid oligomer). Furthermore, the electron-donor molecule(s) and/or electron-acceptor

molecule(s) may be integrated in one or more macromolecule(s), this integration occurring via encapsulation in suitable molecular cavities (binding pockets) of the macromolecule(s), via hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance between the macromolecule(s) and the electron-donor molecule(s) and/or the electron-acceptor molecule(s). Thus, in this case, the macromolecule(s) and the electron-donor molecule(s) and electron-acceptor molecule(s) form the redox-active moiety. If multiple macromolecules are components of the redox-active moiety, the binding of the macromolecules to one another may likewise take place covalently, ionically, via hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance.

[026] According to the present invention, the aforementioned donor and acceptor molecules form a redox-active moiety, i.e. they are bound to one another directly or via further molecular moieties. The sole restriction on the molecules or molecular moieties joining the components of the redox-active moiety, according to the present invention, is the exclusion of nucleic acid oligomers. According to the present invention, the redox-active moiety is bound to the probe oligonucleotide as a complete moiety, multiple chemical bonds being able, of course, to be formed between the oligonucleotide and the redox-active moiety. The exclusion of nucleic acid oligomers as the molecules or molecular moieties joining the components of the redox-active moiety is intended to demonstrate clearly that it is not individual portions of the redox-active moiety that are attached at various sites of the probe oligonucleotide. Thus, the probe oligonucleotide explicitly does not represent the link between the electron-donor molecule(s) or molecular moiety (moieties) and the electron-acceptor molecule(s) or molecular moiety (moieties) of the redox-active moiety.

[027] The redox-active moiety is either a photoinducibly redox-active moiety or a chemically-inducibly redox-active moiety.

[028] In the context of the present invention, "photoinducible" means that the redox activity of the photoinducibly redox-active moiety, in other words its property of giving up electrons to a suitable oxidizing agent or taking up

electrons from a suitable reducing agent under certain external conditions, is exhibited only upon irradiation with light of a specific or any given wavelength. Upon irradiation with light of a specific or any given wavelength, the electron donor "D" gives up an electron to one of the electron acceptors "A" and a charge-separated state D^+A^- is formed, at least temporarily, comprising the oxidized donor and reduced acceptor. This process within the photoinducibly redox-active moiety is referred to as photoinduced charge separation. Given appropriately chosen external conditions, the photoinducibly redox-active moiety exhibits it redox activity only in a charge-separated state, since the reducing agent can transfer (or the oxidizing agent can take up) electrons only to the oxidized donor (or from the reduced acceptor) of the photoinducibly redox-active moiety, for example in the presence of an oxidizing agent that can oxidize A^- but not A (or in the presence of a reducing agent that can reduce D^+ but not D).

[029] In particular, the aforementioned oxidizing or reducing agent may be an electrode, the photoinducibly redox-active moiety being able to give up an electron to the electrode (or take up an electron therefrom) only subsequent to photoinduced charge separation, for example if the electrode is set to a potential at which A^- but not A is oxidized (or D^+ but not D is reduced). In addition, the oxidizing or reducing agent may be a free redox-active substance, the photoinducibly redox-active moiety being able to give up an electron to (or take up an electron from) the free redox-active substance only subsequent to photoinduced charge separation, for example if the free redox-active substance oxidizes A^- but not A (or reduces D^+ but not D).

[030] In the context of the present invention, "chemically inducible" means that the redox activity of the chemically-inducibly redox-active moiety, in other words its property of giving up electrons to a suitable oxidizing agent (or taking up electrons from a suitable reducing agent) under certain external conditions, is exhibited only subsequent to reduction (or oxidation) by an external reducing agent (or oxidizing agent). The chemically-inducibly redox-active moiety corresponds to a photoinducibly redox-active moiety in composition and manner of functioning, but in contrast to the manner in which a photoinducibly redox-active moiety functions, photoactivation is precluded as an external

condition for developing the redox activity of the redox-active moiety. Given appropriately chosen external conditions, the chemically-inducibly redox-active moiety exhibits its redox activity, for example its property of giving up electrons to a suitable oxidizing agent, only after an electron is transferred from a reducing agent to the/an electron donor "D." Only in a reduced state "D^-" can the electron donor transfer an electron to the acceptor "A," and the oxidizing agent can take up electrons only from this reduced acceptor "A^-" of the redox-active moiety, for example in the presence of an oxidizing agent that can oxidize A^- but not A (successive charge transfer). In particular, said oxidizing agent may also be an electrode, for example if the electrode is set to a potential at which A^- but not A is oxidized. Conversely, given differently chosen external conditions, the chemically-inducibly redox-active moiety can exhibit its redox activity, for example its property of taking up electrons from a suitable reducing agent, only after an electron is transferred from an electron acceptor "A" to an oxidizing agent. Only in an oxidized state "A^+" can an electron acceptor take up an electron from the donor D, and the reducing agent can transfer electrons only to the oxidized donor "D^+" of the redox-active moiety, for example in the presence of a reducing agent that can reduce D^+ but not D (successive charge transfer). In particular, said reducing agent may also be an electrode, for example if the electrode is set to a potential at which D^+ but not D is reduced.

[031] In addition to comprising electron donor(s) and electron acceptor(s), or electron donor(s), electron acceptor(s), and macromolecule(s), essential features of the photoinducibly or chemically-inducibly redox-active moiety are: (i) in the forms relevant to the present invention (electron donor(s) and electron acceptor(s) in their original state or in an oxidized or reduced state), the moiety is stable and does not dissociate into its components, (ii) the moiety includes no nucleic acid, (iii) the moiety's composition comprising electron donor(s) and electron acceptor(s) or electron donor(s), electron acceptor(s), and macromolecule(s) can be recognized by a person skilled in the art, regardless of the bond between the components, since, in principle, electron donor(s) and acceptor(s) may also occur as single molecules, and (iv) electron donor(s) and electron acceptor(s) of the redox-active moiety, under

the same or similar external conditions to those of their form that is relevant to the present invention, as components of the redox-active moiety, also act as electron donor(s) and electron acceptor(s) in the form of single molecules in solution, i.e. even in the case of free dissolved electron donor(s) and electron acceptor(s), an electron can be transferred from the dissolved electron donor(s) to the dissolved electron acceptor(s), directly or under the influence of certain external conditions, depending on the conditions that lead to an electron transfer within the redox-active moiety. As in the case of the electron donor(s) and electron acceptor(s) of the photoinducibly redox-active moiety, such an external condition for the free, dissolved electron donor(s) and electron acceptor(s) may be light absorption by the free, dissolved electron donor(s) and acceptor(s), the (an) electron donor "D" giving up an electron to the (an) electron acceptor "A" and a charge-separated state D^+A^- being formed, at least temporarily, comprising a free, dissolved oxidized donor and a free, dissolved reduced acceptor. Another such external condition may be – as with the electron donor(s) and electron acceptor(s) of the chemically-inducibly redox-active moiety – the transfer of an electron to the free, dissolved electron donor by a reducing agent or the release of an electron to an oxidizing agent by the free, dissolved electron acceptor.

[032] The photoinducibly redox-active moiety may be for example any photoinducibly redox-active protein/enzyme or any photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex. In the expression "photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex," the term "at least bimolecular" means that the complex is composed of at least one electron donor and at least one electron acceptor, even if this donor and this acceptor are directly (or indirectly via a spacer) covalently joined. Upon irradiation with light of a specific or any given wavelength, the/an electron donor gives up an electron to one of the electron acceptors and a charge-separated state D^+A^- is formed, at least temporarily, comprising an oxidized donor and a reduced acceptor. This process within the photoinducibly redox-active moiety is referred to as photoinduced charge separation. Given appropriately chosen external conditions, the photoinducibly redox-active moiety exhibits its redox activity, in other words its property of giving up electrons to a suitable oxidizing agent or

taking up electrons from a suitable reducing agent, only in a charge-separated state, since the reducing agent transfers (or the oxidizing agent takes up) electrons only to the oxidized donor (or from the reduced acceptor) of the photoinducibly redox-active moiety, for example in the presence of a reducing agent that can reduce D^+ but not D (or in the presence of an oxidizing agent that can oxidize A^- but not A). In particular, this oxidizing or reducing agent may also be an electrode, the photoinducibly redox-active moiety being able to give up an electron to an electrode (or take up an electron therefrom) only subsequent to photoinduced charge separation, for example if the electrode is set to a potential at which A^- but not A is oxidized (or D^+ but not D is reduced).

[033] The chemically-inducibly redox-active moiety may be for example any chemically-inducibly redox-active protein/enzyme or any chemically-inducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex. In the expression "chemically-inducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex," the term "at least bimolecular" means that the complex is composed of at least one electron donor and at least one electron acceptor, even if this donor and this acceptor are directly (or indirectly via a spacer) covalently joined. Given appropriately chosen external conditions, the chemically-inducibly redox-active moiety exhibits its redox activity, in other words its property of giving up electrons to a suitable oxidizing agent (or taking up electrons from a suitable reducing agent) under certain external conditions, only subsequent to reduction (or subsequent to oxidation) by an external reducing agent (or oxidizing agent). Only after an electron is transferred from a reducing agent to the/an electron donor "D" can the then-reduced donor "D^-" transfer an electron to the acceptor "A," and the oxidizing agent can take up electrons only from this reduced acceptor "A^-" of the redox-active moiety. In particular, the aforementioned oxidizing agent may also be an electrode, for example if the electrode is set to a potential at which A^- but not A is oxidized. Conversely, given differently chosen external conditions, only after an electron is transferred from the acceptor "A" to an external oxidizing agent can the electron acceptor of the chemically-inducibly redox-active moiety, in its then-oxidized state "A^+," take up an electron from the donor D and the reducing agent can transfer electrons only to the oxidized

donor "D^+" of the redox-active moiety. In particular, the aforementioned reducing agent may also be an electrode, for example if the electrode is set to a potential at which D^+ but not D is reduced.

[034] In the context of the present invention, the term "oxidizing agent" refers to a chemical compound (chemical substance) that, by taking up electrons from another chemical compound (chemical substance, electron donor, electron acceptor), oxidizes this other chemical compound (chemical substance, electron donor, electron acceptor). The oxidizing agent behaves analogously to an electron acceptor but is used in the context of the present invention to refer to an external electron acceptor not directly belonging to the redox-active moiety. In this context, "not directly" means that the oxidizing agent is either a free redox-active substance that is not bound to but in contact with the nucleic acid oligomer, or that the oxidizing agent is covalently attached to the nucleic acid oligomer, but at a nucleic acid oligomer site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the redox-active moiety. In particular, the electrode may represent the oxidizing agent.

[035] Within the context of the present invention, the term "reducing agent" refers to a chemical compound (chemical substance) that, by giving up electrons to another chemical compound (chemical substance, electron donor, electron acceptor), reduces this other chemical compound (chemical substance, electron donor, electron acceptor). The reducing agent behaves analogously to an electron donor but is used in the context of the present invention to refer to an external electron donor not directly belonging to the redox-active moiety. In this context, "not directly" means that the reducing agent is either a free redox-active substance that is not bound to but in contact with the nucleic acid oligomer, or that the reducing agent is covalently attached to the nucleic acid oligomer, but at a nucleic acid oligomer site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the redox-active moiety. In particular, the electrode may represent the reducing agent.

[036] In the context of the present invention, the term "free redox-active substance" refers to a free oxidizing or reducing agent not covalently joined with but

in contact with the redox-active moiety, the nucleic acid oligomer, or the conductive surface, for example via the solution applied to the modified conductive surface, the free redox-active substance being able to be for example an uncharged molecule, any salt, or a redox-active protein or enzyme (oxido-reductase). The free redox-active substance is characterized in that it can rereduce the oxidized donor (or reoxidize the reduced acceptor) of the photoinducibly redox-active moiety, or in that the free redox-active substance can reduce the donor (or oxidize the acceptor) of the chemically-inducibly redox-active moiety. Furthermore, the free redox-active substance is characterized in that it is oxidizable and reducible at a potential φ, where φ satisfies the condition $2.0\ V \geq \varphi \geq -2.0\ V$. The potential refers here to the free redox-active molecule in a suitable solvent, measured against a normal hydrogen electrode. In the context of the present invention, the potential range $1.7\ V \geq \varphi \geq -1.7\ V$ is preferred, the range $1.4\ V \geq \varphi \geq -1.2\ V$ being particularly preferred and the range $0.9\ V \geq \varphi \geq -0.7\ V$, at which the redox-active substances of the application examples are oxidized (or reduced), being most particularly preferred.

[037] The modified nucleic acid oligomer is directly or indirectly (via a spacer) bound to a conductive surface. The term "conductive surface" is understood to mean any electrically conductive surface of any thickness, especially metallic surfaces, surfaces comprising metal alloys, or doped or non-doped semiconductor surfaces, all semiconductors being able to be used in the form of pure substances or in the form of mixtures. In the context of the present invention, the conductive surface may be present alone or applied to any support material such as glass. In the context of the present invention, the term "electrode" is used as an alternative to "conductive surface."

[038] The term "modified conductive surface" is understood to mean a conductive surface that is modified by attaching a nucleic acid oligomer modified with a redox-active moiety.

[039] According to a further aspect, the present invention is directed to a method that allows the electrochemical detection of molecular structures, in particular the electrochemical detection of DNA/RNA/PNA fragments in a probe solution by sequence-specific nucleic acid oligomer hybridization. The detection of the hybridization events via electrical signals is a simple and

economical method and, in a battery-operated variation, allows on-site application.

[040] The present invention further provides a photoaddressable read-out method for detecting molecular structures, inter alia for detecting hybridization events on an oligomer chip, for example via electrical signals. According to the present invention, a "photoaddressable (oligomer chip) read-out method" is understood to be a method in which the detection of molecular structures is limited to a specific test site or group of test sites within the entire system (of the complete oligomer chip) by light of a specific or any given wavelength being spatially focused on (limited to) this test site (group) in order to induce the redox activity of the photoinducibly redox-active moiety.

[041] **Binding a Redox-Active Moiety to a Nucleic Acid Oligomer**

[042] A prerequisite for the method according to the present invention is the binding of a photoinducibly redox-active moiety or a chemically-inducibly redox-active moiety to a nucleic acid oligomer.

[043] The following are some examples of a photoinducibly redox-active moiety:

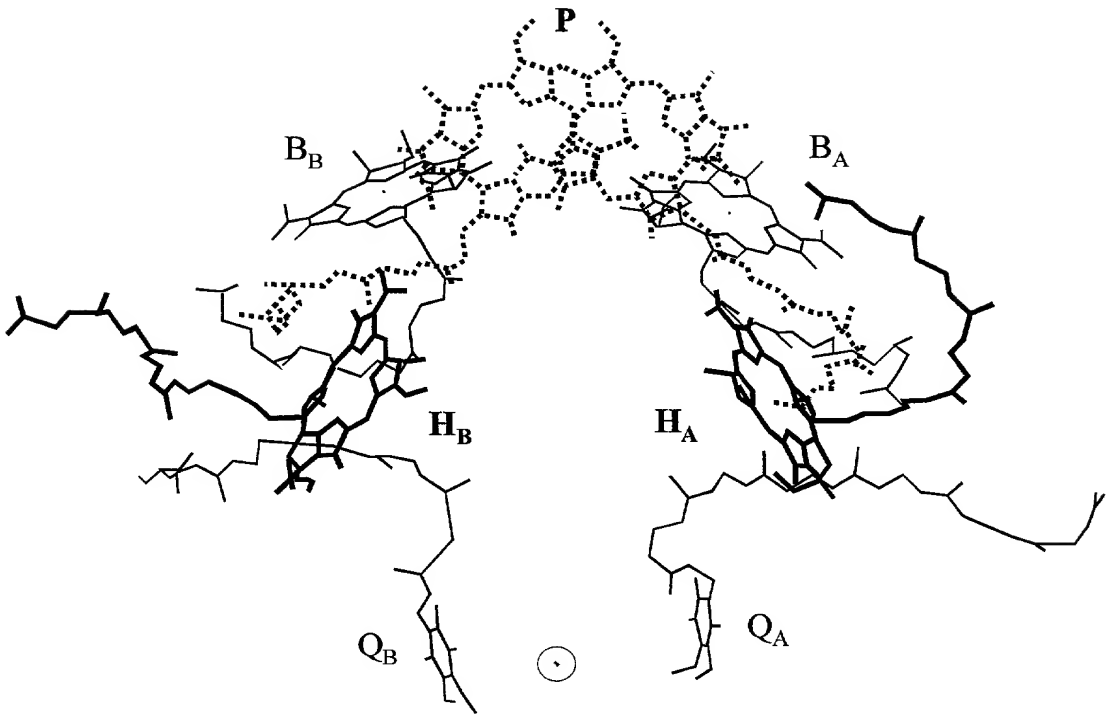
[044] (i) The photosynthetic bacterial reaction center (RC), such as the RC of *Rhodobacter sphaeroides* having the schematic Structure 1; the RC of other photosynthetic bacteria, such as the reaction center of *Rhodopseudomonas viridis* or *Rhodobacter capsulatus*; or a reaction center of photosynthesizing plants, such as photosystem 1 or photosystem 2, as examples of a photoinducibly redox-active protein/enzyme.

[045] (ii) Cyclophanes, or bridged porphyrin quinone systems, having the general Structure 2, as an example of a photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex. The two spacer-bridged covalent links ("- - spacer - -" in Structure 2) between the electron acceptor (1,4-benzoquinone in Structure 2) and the electron donor (metalloporphyrin in Structure 2) may be affixed to any site on the electron donor and/or electron acceptor. In addition to the electron acceptors shown in Structure 2, flavins having the general Formula 1, nicotinamides having the general Formula 2 or other quinones, e.g. those having the general

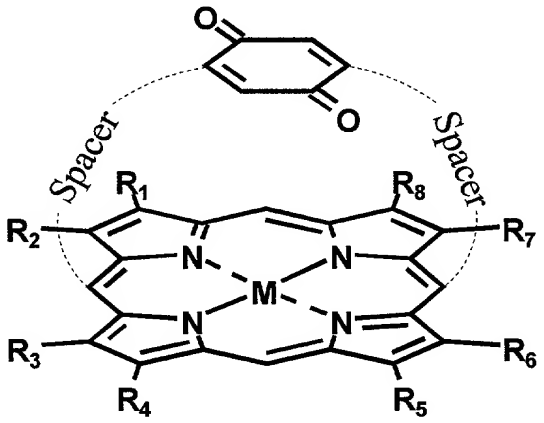
Formulas 3 – 8, or organic or inorganic electron acceptors and, moreover, in addition to the (metallo)porphyrins having the general Formula 9, other electron donors such as (metallo)chlorophylls having the general Formula 10 or (metallo)bacteriochlorophylls having the general Formula 11, or other organic or inorganic electron donors may also be used. In addition, simple covalently (spacer-)bridged electron-donor/electron-acceptor complexes such as covalent compounds of a substance according to Formula 9 and one of the substances according to one of Formulas 1 – 8, covalent compounds of a substance according to Formula 10 and one of the substances according to one of Formulas 1 – 8, or covalent compounds of a substance according to Formula 11 and one of the substances according to one of Formulas 1 – 8 may be used as photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complexes.

[046] (iii) Photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complexes in which (one of) the electron donor(s) and/or (one of) the electron acceptor(s) is a charge transfer complex or transition metal complex. Examples of transition metal complexes are $[Ru(bipy)_2(py)(im)]^{2+}$, any other [Ru(II)(L1)(L2)(L3)(L4)(L5)(L6)] complexes, Cr(III), Fe(II), Os(II), or Co(II) complexes, wherein "bipy" stands for a bispyridyl ligand, "py" for a pyridyl ligand, "im" for an imidazole ligand, and L1 to L3 for any ligand, and also more or fewer than 6 ligands may coordinate on a transition metal.

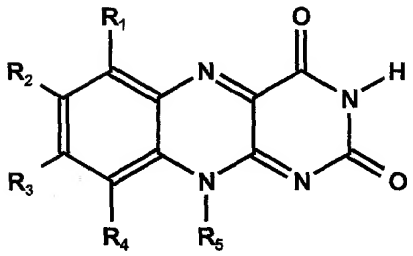
[047] Examples of a chemically-inducibly redox-active moiety are the cytochrome-bc complex or the cytochrome-c_2 complex of photosynthesizing bacteria (a complex comprising a protein matrix and four embedded iron-porphyrin cofactors as electron donors and/or electron acceptors), as examples of a chemically-inducibly redox-active protein/enzyme or, as listed under (ii) and (iii), suitably composed cyclophanes or analogous compounds, as examples of a chemically-inducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex.



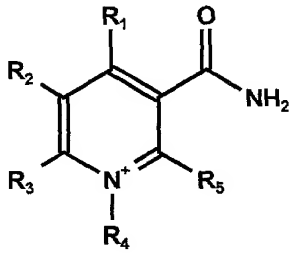
[048] *Structure 1*: Reaction center consisting of the cofactors P (primary donor, a bacteriochlorophyll dimer), B_A and B_B (bacteriochlorophyll monomers), H_A and H_B (bacteriopheophytins), Q_A and Q_B (ubiquinone-50) and the protein sub-units L, M, and H (not shown) that envelop the cofactors.



[049] *Structure 2*: A cyclophane; M = e.g. 2H, Mg, Zn, Cu, Ni, Pd, Co, Cd, Mn, Fe, Sn, Pt, etc.; R_1 to R_8, or spacers, are, independently of one another, any alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, or heteroalkynyl substituents.



Formula 1



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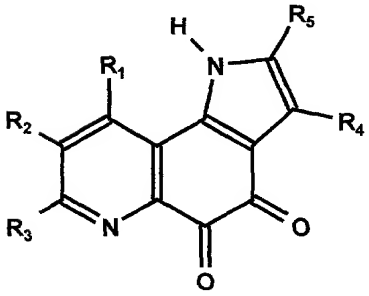

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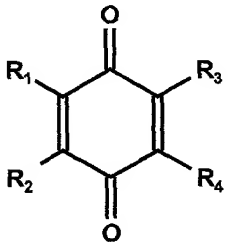

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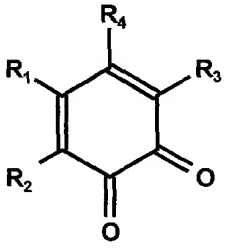

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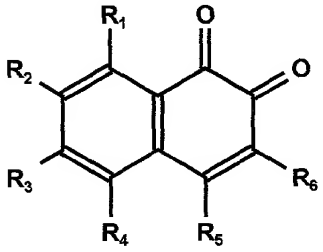

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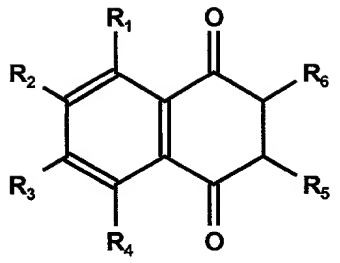

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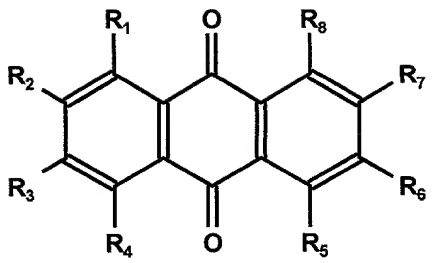
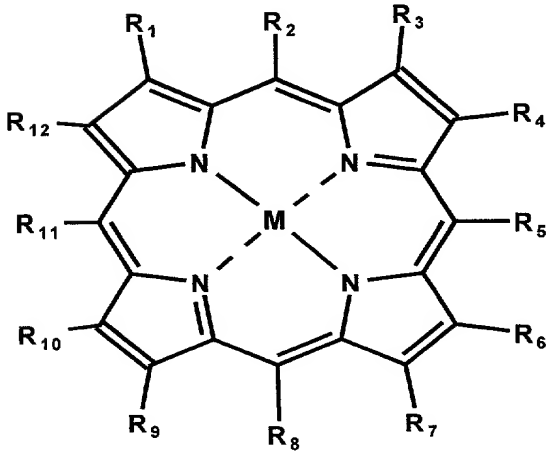


Formula 8

[050] R_1 to R_8 are, independently of one another, H or any alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, or heteroalkynyl substituents.



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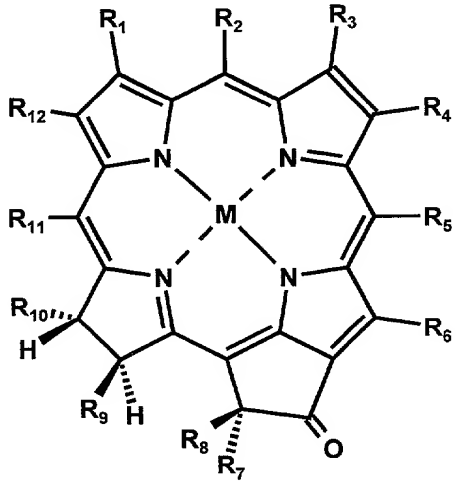
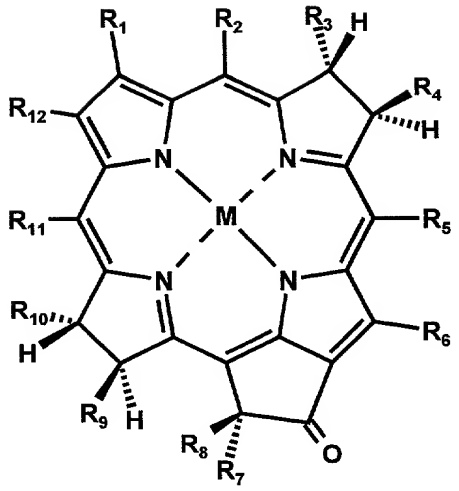


Formula 10



Formula 11

[051] M = 2H, Mg, Zn, Cu, Ni, Pd, Co, Cd, Mn, Fe, Sn, Pt, etc.; R_1 to R_{12} are, independently of one another, H or any alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, or heteroalkynyl substituents.

[052] In addition, according to the present invention, a distinguishing feature of the redox-active moiety is that said moiety gives up electrons to an oxidizing agent that is likewise covalently attached to the nucleic acid oligomer, or takes up electrons from another reducing agent that is likewise covalently attached to the oligonucleotide, this oxidizing or reducing agent being able to be in particular an electrically conductive surface (electrode) and the redox-active moiety being able be electrooxidized/electroreduced by applying an external voltage to this electrode in its electrochemically accessible potential range.

[053] According to the present invention, a distinguishing feature of the redox-active substance is that it can rereduce the photoinducibly redox-active moiety (or can reoxidize it) after the latter gives up an electron to another oxidizing agent that is distinct from the redox-active substance and covalently attached to the oligonucleotide (or after the latter takes up an electron from another reducing agent that is distinct from the redox-active substance and covalently attached to the oligonucleotide), or that the free redox-active substance can reduce (or oxidize) the donor (or the acceptor) of the chemically-inducibly redox-active moiety. According to the present invention, any redox-active

substance may be used for this as long as it is oxidizable and reducible at a potential φ that satisfies the condition 2.0 V $\geq \varphi \geq$ - 2.0 V and as long as the potential is suitable for rereducing (or reoxidizing) said photoinducibly redox-active moiety after the latter gives up an electron to another oxidizing agent that is likewise covalently attached to the nucleic acid oligomer (or takes up an electron from another reducing agent that is likewise covalently attached to the nucleic acid oligomer), or for reducing or oxidizing said chemically-inducibly redox-active moiety. The potential refers here to the free, unmodified redox-active substance in a suitable solvent, measured against a normal hydrogen electrode. In the context of the present invention, the potential range 1.7 V $\geq \varphi \geq$ - 1.7 V is preferred, the range 1.4 V $\geq \varphi \geq$ - 1.2 V being particularly preferred and the range 0.9 V $\geq \varphi \geq$ - 0.7 V, in which the redox-active substances of the application example are oxidized (and rereduced), being most particularly preferred. Suitable possibilities are, in addition to the usual organic and inorganic redox-active molecules such as hexacyanoferrates, ferrocenes, cobaltocenes, and quinones, most importantly ascorbic acid (or the Na^+ salt thereof), $[Ru(NH_3)_6]^{2+}$, or cytochrome c_2 $(cyt\ c_2)^{2+}$, a freely movable iron-containing protein that reduces the oxidized primary donor P^+ in the RC of *Rhodobacter sphaeroides* to P and, in doing so, is itself oxidized to $(cyt\ c_2)^{3+}$.

[054] In a preferred embodiment of the present invention, the photoinducibly or chemically-inducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex is embedded in one or more macromolecules in such a way that the macromolecule acts as an electrically insulating, enveloping end of the redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex by preventing direct electrooxidation/electroreduction of the redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex at the electrode, for example in the case of direct contact between the electrode and the redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex, but allowing indirect electrooxidation/electroreduction of the electron-donor/electron-acceptor complex mediated by a double-stranded nucleic acid oligomer. Such a macromolecule can be for example a tailored cyclodextrin

that, due to its being shaped as a cut-off cone that is hollow inside, coats a cyclophane or similar electron-donor/electron-acceptor complex.

[055] According to the present invention, a redox-active moiety is covalently bound to a nucleic acid oligomer by the reaction of the nucleic acid oligomer with the redox-active moiety or portions thereof (see also the section "Manner of Executing the Invention"). This bond can be achieved in four different ways:

[056] a) A free phosphoric-acid, sugar-C-3-hydroxy, carboxylic-acid, or amine group of the oligonucleotide backbone, especially a group at one of the two ends of the oligonucleotide backbone, is used as the reactive group for forming a bond at the nucleic acid oligomer. The free, terminal phosphoric-acid, sugar-C-3-hydroxy, carboxylic-acid, or amine groups exhibit increased reactivity and thus easily undergo typical reactions such as amidation with (primary or secondary) amino groups or with acid groups; esterification with (primary, secondary, or tertiary) alcohols or with acid groups; thioester formation with (primary, secondary, or tertiary) thioalcohols or with acid groups, or condensation of amine and aldehyde with subsequent reduction of the resultant CH=N bond to a CH_2-NH bond. The coupling group (acid, amine, alcohol, thioalcohol, or aldehyde function) required to covalently attach the redox-active moiety is either naturally present on the redox-active moiety or is obtained by chemically modifying the redox-active moiety. The attachment of the redox-active moiety may take place completely or in portions of the moiety with subsequent completion of the redox-active moiety (see below).

[057] b) The nucleic acid oligomer is modified with a reactive group at the oligonucleotide backbone or at a base via a covalently-attached molecular moiety (spacer) of any composition and chain length (longest continuous chain of atoms bound to one another), especially a chain length of 1 to 14. The modification preferably takes places at one of the ends of the oligonucleotide backbone or at a terminal base. An alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, or heteroalkynyl substituent, for example, may be used as the spacer. Possible simple reactions for forming the covalent bond between the redox-active moiety and the nucleic acid oligomer thus modified are, as described under a), amidation from an acid and amino group, esterification from an acid and alcohol group, thioester formation from an acid and

thioalcohol group, or condensation of aldehyde and amine with subsequent reduction of the resultant CH=N bond to a CH_2-NH bond. The attachment of the redox-active moiety may take place completely or in portions of the redox-active moiety with subsequent completion of the moiety (see below).

[058] c) If the nucleic acid oligomer is synthesized, a terminal base will be replaced by the redox-active moiety. This attachment of the redox-active moiety may take place completely or in portions of the moiety with subsequent completion of the redox-active moiety (see below).

[059] d) If a linked (at least bimolecular) electron-donor/electron-acceptor complex is used as the redox-active moiety, the electron acceptor (or donor) will, in a first covalent modification, as described under b) or c) in this section, be bound to a terminal base, or in place of a terminal base, to the nucleic acid oligomer and thereafter, in a second covalent modification of the electron donor (or acceptor), as described under a) in this section, bound at the same end of the nucleic acid oligomer backbone to a reactive group of the backbone or to a reactive group of the acceptor (or donor). If a covalently-linked, trimolecular or greater electron-donor/electron-acceptor complex is used, then, instead of the electron acceptor (or donor), any portion of the electron-donor/electron-acceptor complex may also be used in the first covalent modification and completed in a second or further covalent modification(s).

[060] According to the present invention, the binding of the redox-active moiety to the nucleic acid oligomer may take place completely or in portions, before or after the nucleic acid oligomer is bound to the conductive surface. Thus, in the case of a redox-active protein/enzyme comprising apoprotein and cofactor(s), instead of the complete redox-active moiety, it is also possible for only the apoprotein, the apoprotein and a portion of the cofactors, or one or more cofactors to be attached and the redox-active moiety will be completed by subsequent reconstitution with the remaining missing portions. If a linked (at least bimolecular) electron-donor/electron-acceptor complex is used as the redox-active moiety, the electron acceptor (or donor) will, in a first covalent modification, as described under b) or c) in this section, be bound to a terminal base, or in place of a terminal base, to the nucleic acid oligomer and thereafter, in a second covalent modification of the electron donor (or

acceptor), as described under a) in this section, bound at the same end of the nucleic acid oligomer backbone to a reactive group of the backbone. If a covalently-linked, trimolecular or greater electron-donor/electron-acceptor complex is used, then, instead of the electron acceptor (or donor), any portion of the electron-donor/electron-acceptor complex may also be used in the first covalent modification and completed in the second covalent modification. These modifications may take place before or after the nucleic acid oligomer is bound to the conductive surface.

[061] If there are multiple different nucleic acid oligomer combinations (test sites) on a shared surface, and the redox-active moiety is to be attached to the surface after the nucleic acid oligomer is immobilized, it is advantageous to standardize the (covalent) attachment of the redox-active moiety to the nucleic acid oligomers for the entire surface by the appropriate choice of reactive group at the free nucleic acid oligomer ends of the various test sites.

[062] If redox-active proteins/enzymes are used as the redox-active moiety, the covalent attachment of the nucleic acid oligomer may take place at any reactive group that is naturally present on or affixed to the protein by modification, or – in the event that the redox-active protein/enzyme consists of apoprotein and cofactor(s) – at any reactive group that is naturally present on or affixed to a (any) cofactor by modification. In the context of the present invention, the covalent attachment at any reactive group that is naturally present on or affixed by modification to a (any) cofactor of the protein is preferred. Without wanting to be bound to mechanistic details, if there are multiple cofactors, special preference is given to the one that can give up electrons to an external oxidizing agent that is likewise covalently attached to the nucleic acid oligomer, or that can take up electrons from an external reducing agent that is likewise covalently attached to the nucleic acid oligomer (see also the section "Method of Amperometrically Detecting Nucleic Acid Oligomer Hybrids").

[063] **The Conductive Surface**

[064] According to the present invention, the term "conductive surface" is understood to mean any support having an electrically conductive surface of

any thickness, especially surfaces comprising platinum, palladium, gold, cadmium, mercury, nickel, zinc, carbon, silver, copper, iron, lead, aluminum, and manganese.

[065] In addition, any doped or non-doped semiconductor surfaces of any thickness may also be used. All semiconductors may be used in the form of pure substances or in the form of mixtures. Examples include, but are not limited to, carbon, silicon, germanium, α tin, and Cu(I) and Ag(I) halides of any crystal structure. Also suitable are all binary compounds of any composition and any structure comprising the elements of groups 14 and 16, the elements of groups 13 and 15, and the elements of groups 15 and 16. In addition, ternary compounds of any composition and any structure comprising the elements of groups 11, 13, and 16 or the elements of groups 12, 13, and 16 may be used. The designations of the groups of the periodic system refer to the IUPAC recommendation of 1985.

[066] **Binding a Nucleic Acid Oligomer to the Conductive Surface**

[067] According to the present invention, a nucleic acid oligomer is linked directly or via a linker/spacer with the surface atoms or molecules of a conductive surface of the type described above. This binding may be carried out in three different ways:

[068] a) The surface is modified in such a way that a reactive molecule group is accessible. This may take place by direct derivatization of the surface molecules, for example by wet chemical or electrochemical oxidation/reduction. Thus, for example, the surface of graphite electrodes can be provided with aldehyde or carboxylic-acid groups by wet chemical oxidation. Electrochemically, it is possible, for example by reduction in the presence of aryl-diazonium salts, to couple the appropriate (functionalized, i.e. provided with a reactive group) aryl radical, or by oxidation in the presence of $R'CO_2H$, to couple the (functionalized) R'-radical to the graphite electrode surface. An example of direct modification of semiconductor surfaces is the derivatization of silicon surfaces to reactive silanols, i.e. silicon supports having Si-OR" groups on the surface, both R" and R' representing any functionalized organic residue (e.g. alkyl, alkenyl, alkynyl, heteroalkyl,

heteroalkenyl, or heteroalkynyl substituent). Alternatively, the entire surface may be modified by covalently attaching a reactive group of a bifunctional linker, such that a monomolecular layer comprising any molecules and including a reactive group, preferably terminally, results on the surface. The term "bifunctional linker" is understood to mean any molecule of any chain length, especially chain lengths 2 – 14, having two identical (homobifunctional) or two different (heterobifunctional) reactive molecule groups.

[069] If multiple different test sites are to be formed on the surface by making use of the methodology of photolithography, then at least one of the reactive groups of the homo- or heterobifunctional linkers is a photoinducibly reactive group, i.e. a group that becomes reactive only upon irradiation with light of a specific or any given wavelength. This linker is applied in such a way that the/a photoactivatable reactive group is available after the linker is covalently attached to the surface. The nucleic acid oligomers are covalently attached to the surface thus modified, and are themselves modified with a reactive group via a spacer of any composition and chain length, especially chain lengths 1 – 14, preferably near an end of the nucleic acid oligomer. The reactive group of the oligonucleotide is one of any of the groups that react directly (or indirectly) with the modified surface to form a covalent bond. In addition, a further reactive group may be bound to the nucleic acid oligomers near their second end, this reactive group, in turn, being attached, as described above, directly or via a spacer of any composition and chain length, especially a chain length of 1 – 14. Furthermore, as an alternative to this further reactive group, the redox-active moiety (completely or portions thereof) may be attached to this second end of the nucleic acid oligomer.

[070] b) The nucleic acid oligomer that is to be applied to the conductive surface is modified with one or more reactive groups via a covalently-attached spacer of any composition and chain length, especially a chain length of 1 – 14, the reactive groups being located preferably near an end of the nucleic acid oligomer. The reactive groups are groups that can react directly with the unmodified surface. Some examples are: (i) thiol- (HS-) or disulfide- (S-S-) derivatized nucleic acid oligomers having the general formula (n x HS-spacer)-oligo, (n x R-S-S-spacer)-oligo, or oligo-spacer-S-S-spacer-oligo that react

with a gold surface to form gold-sulfur bonds or (ii) amines that attach to platinum or silicon surfaces by chemisorption or physisorption. In addition, a further reactive group may be bound to the nucleic acid oligomers near their second end, this reactive group, in turn, being attached, as described above, directly or via a spacer of any composition and chain length, especially a chain length of 1 – 14. Furthermore, as an alternative to this further reactive group, the photoinducibly redox-active moiety (completely or portions thereof) may be attached at this second end of the oligonucleotide. Particularly nucleic acid oligomers that are modified with multiple spacer-bridged thiol or disulfide bridges ((n x HS-spacer)-oligo or (n x R-S-S-spacer)-oligo) have the advantage that such nucleic acid oligomers can be applied to the conductive surface at a specific setting angle (angle between the surface normal and the helix axis of a double-stranded helical nucleic acid oligomer or between the surface normal and the axis perpendicular to the base pairs of a double-stranded non-helical nucleic acid oligomer) if the spacers attaching the thiol or disulfide functions to the nucleic acid oligomer possess an increasing or decreasing chain length as viewed from an end of the nucleic acid.

[071] c) Groups used as the reactive group on the probe nucleic acid oligomer are phosphoric-acid, sugar-C-3-hydroxy, carboxylic-acid, or amine groups of the oligonucleotide backbone, especially terminal groups. The phosphoric-acid, sugar-C-3-hydroxy, carboxylic-acid, or amine groups exhibit increased reactivity and consequently enter easily into typical reactions such as amidation with (primary or secondary) amino or acid groups, esterification with (primary, secondary, or tertiary) alcohols or acid groups, thioester formation with (primary, secondary, or tertiary) thioalcohols or acid groups, or condensation of amine and aldehyde with subsequent reduction of the resultant CH=N bond to a CH_2-NH bond. In this case, the coupling group required for covalent attachment to the phosphoric-acid, sugar-C-3-hydroxy, carboxylic-acid, or amine group is part of the surface derivatization with a (monomolecular) layer having any molecule length, as described under a) in this section, or the phosphoric-acid, sugar-C-3-hydroxy, carboxylic-acid, or amine group can react directly with the unmodified surface, as described under b) in this section. In addition, a further reactive group may be bound to

the oligonucleotides near their second end, this reactive group, in turn, being attached, as described above, directly or via a spacer of any composition and chain length, especially a chain length of 1 – 14. Furthermore, as an alternative to this further reactive group, the redox-active moiety (completely or portions thereof) may be attached to this second end of the nucleic acid oligomer.

[072] Binding the nucleic acid oligomer to the conductive surface may take place before or after the redox-active moiety is attached to the nucleic acid oligomer. In the case of a redox-active protein/enzyme comprising apoprotein and cofactor(s), instead of the complete redox-active moiety, it is also possible for only the apoprotein, the apoprotein and a portion of the cofactors, or one or more of the cofactors to be attached and the redox-active moiety will be completed by subsequent reconstitution with the remaining missing portions. If a linked (at least bimolecular) electron-donor/electron-acceptor complex is used as the redox-active moiety, the electron acceptor (or donor) may, as described under b) or c) in the section "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," be attached to a terminal base, or in place of a terminal base, to the nucleic acid oligomer, and the electron donor (or acceptor) may be attached by subsequent covalent attachment to a reactive group of the electron acceptor (or donor) or, as described under a) in the section "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," by subsequent attachment to a terminal reactive group of the nucleic acid oligomer backbone at the same end (see also the section "Manner of Executing the Invention"). Alternatively, binding the nucleic acid oligomer to the conductive surface may take place before or after the spacer having a reactive group for binding the redox-active moiety is attached. Binding the already modified nucleic acid oligomer to the conductive surface, i.e. binding to the surface after the redox-active moiety is attached to the nucleic acid oligomer or after portions of the redox-active moiety are attached, or after the spacer having a reactive group for binding the redox-active moiety is attached, likewise takes place as described under a) to c) in this section.

[073] In producing the test sites, when attaching the single-strand nucleic acid oligomers to the surface, care must be taken that sufficient distance

remains between the individual nucleic acid oligomers to provide, first, the space necessary for hybridization with the target nucleic acid oligomer and, second, the space necessary for the attachment of the redox-active moiety. Three different methods of proceeding (and combinations thereof) offer themselves for this purpose:

[074] 1.) Producing a modified surface by attaching a hybridized nucleic acid oligomer, in other words a surface derivatization with hybridized probe nucleic acid oligomer instead of with single-strand probe oligonucleotide. The nucleic acid oligomer strand used for hybridization is unmodified (the surface attachment is carried out as described under a) - c) in this section). Thereafter, the hybridized nucleic acid oligomer double-strand is thermally dehybridized, thus producing a single-strand-nucleic-acid-oligomer-modified surface having greater distance between the probe nucleic acid oligomers.

[075] 2.) Producing a modified surface by attaching a single-strand or double-strand nucleic acid oligomer, adding, during surface derivatization, a suitable monofunctional linker that, in addition to the single-strand or double-strand nucleic acid oligomer, is also bound to the surface (the surface attachment is carried out as described under a) – c) in this section). According to the present invention, the monofunctional linker has a chain length that is identical to the chain length of the spacer between the surface and the nucleic acid oligomer, or that differs by a maximum of four chain atoms. If double-strand nucleic acid oligomer is used for surface derivatization, the nucleic acid oligomer double-strand is thermally dehybridized after the double-strand nucleic acid oligomer and the linker are jointly attached to the surface. By simultaneously attaching a linker to the surface, the distance between the single-strand or double-strand nucleic acid oligomers that are likewise bound to the surface is increased. If a double-strand nucleic acid oligomer is used, this effect is amplified further by the subsequent thermal dehybridization.

[076] 3.) Producing a modified surface by attaching a single-strand or double-strand oligonucleotide to which the redox-active moiety is already attached, the redox-active moiety having a diameter of greater than 30 Å. If double-strand oligonucleotide is used, the oligonucleotide double-strand is thermally dehybridized after the double-strand oligonucleotide is attached to the surface.

[077] Regarding the individual steps in "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer," as well as in "Binding an Oligonucleotide to the Conductive Surface," it should be noted that, in the section "Manner of Executing the Invention," the various combination possibilities of the individual steps that lead to the same end result are demonstrated in an example (Figure 2).

[078] **Method of Electrochemically Detecting Nucleic Acid Oligomer Hybrids**

[079] Advantageously, according to the method of electrochemically detecting nucleic acid oligomer hybrids, multiple probe nucleic acid oligomers varying in sequence, ideally all necessary combinations of the nucleic acid oligomer, are applied to an oligomer (DNA) chip to detect the sequence of any target nucleic acid oligomer or (fragmented) target DNA, or in order to seek and sequence-specifically detect mutations in the target. For this purpose, the surface atoms or molecules of a defined area (a test site) on a conductive surface are linked with DNA/RNA/PNA nucleic acid oligomers having a known but arbitrary sequence, as described above. In a most general embodiment, however, the DNA chip may also be derivatized with a single probe oligonucleotide. Preferred probe nucleic acid oligomers are nucleic acid oligomers (e.g. DNA, RNA, or PNA fragments) of base length 3 to 50, preferably of length 5 to 30, particularly preferably of length 8 to 25. According to the present invention, a redox-active moiety is or becomes bound to the probe nucleic acid oligomers, as described below.

[080] The modification of the probe nucleic acid oligomers with a redox-active moiety may take place completely or in components of the redox-active moiety, either before or after the oligonucleotide probe is bound to the conductive surface. The various combination possibilities of the individual steps (reaction sequences) are demonstrated in the section "Manner of Executing the Invention" with the aid of Figure 2 using the example of a redox-active moiety bound to an electrode via a probe oligonucleotide.

[081] Regardless of the respective reaction sequence, a surface hybrid having the general structure elec-spacer-ss-oligo-spacer-moiety develops, "moiety" representing the photoinducibly or chemically-inducibly redox-active moiety. The bridges may, of course, also be produced without spacers or with

only one spacer (elec-ss-oligo-spacer-moiety or elec-spacer-ss-oligo-moiety). In the example in Figure 2, the moiety is a photoinducibly redox-active moiety, namely the reaction center (RC) of the photosynthesizing bacteria of the strain *Rhodobacter sphaeroides*, a photoinducibly redox-active protein consisting of apoprotein and cofactors. In the example in Figures 2, 3, and 4, the RC, via its cofactor ubiquinone-50 (UQ) in what is known as the Q_A protein binding pocket of the RC, is covalently joined with the nucleic acid oligomer. The RC forms a 1:1 complex with the cofactor ubiquinone-50 in the Q_A binding pocket, the ubiquinone-50 being covalently bound to the nucleic acid oligomer in the manner described. In the example in Figures 5 and 6, the moiety is a photoinducibly redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex, namely a covalently-linked zinc-bacteriochlorophyll-quinone complex that is covalently joined (via a spacer) with the nucleic acid oligomer via the quinone, the electron-acceptor molecule of the complex.

[082] The electrochemical communication between the (conductive) surface and the redox-active moiety ("moiety") bridged via a single-strand oligonucleotide having the general structure elec-spacer-ss-oligo-spacer-moiety is weak or nonexistent.

[083] In a next step, the test sites are brought into contact with the nucleic acid oligomer solution to be examined (target). This leads to hybridization only if the solution contains nucleic acid oligomer strands that are complementary to the probe nucleic acid oligomers bound to the conductive surface, or complementary in at least wide areas. Hybridization between the probe and target nucleic acid oligomers leads to increased conductivity between the surface and the redox-active moiety, since the latter is now bridged via the nucleic acid oligomer consisting of a double-strand. Figure 3 illustrates this schematically using elec-spacer-ss-oligo-spacer-UQ(RC) as an example. In Figure 4, the sequence of the electron transfer steps in elec-spacer-ds-oligo-spacer-UQ(RC) is shown in detail, while Figure 5 schematically illustrates the example elec-spacer-ss-oligo-spacer-Q-ZnBChl and Figure 6 shows in detail the sequence of the electron transfer steps in elec-spacer-ds-oligo-spacer-Q-ZnBChl.

[084] As a result of the hybridization of the probe nucleic acid oligomer and the nucleic acid oligomer strand that is complementary thereto (target), the electrical communication between the (conductive) surface and the photoinducibly redox-active moiety changes. Thus, a sequence-specific hybridization event can be detected by electrochemical methods such as cyclic voltammetry, amperometry, or conductivity measurements.

[085] In cyclic voltammetry, the potential of a stationary working electrode is changed linearly as a function of time. Starting at a potential at which no electrooxidation or electroreduction occurs, the potential is changed until the redox-active substance is oxidized or reduced (i.e., a current flows). After running through the oxidation or reduction operation, which produces in the current-voltage curve an initially increasing current, then a maximum current (peak), and finally a gradually decreasing current, the direction of the potential feed is reversed. The behavior of the products of electrooxidation or electroreduction is then recorded in a reverse run.

[086] An alternative electrical detection method, amperometry, is made possible by applying a suitable constant electrode potential such that the redox-active moiety may be electrooxidized (electroreduced), but the rereduction (reoxidation) of the redox-active moiety to its original state takes place, not by changing the electrode potential as in cyclic voltammetry, but rather by adding a suitable reducing agent (oxidizing agent), the "redox-active substance," to the target solution, thereby closing the current circuit of the entire system. As long as such a reducing agent (oxidizing agent) is present, or as long as the consumed reducing agent (oxidizing agent) is rereduced (reoxidized) on the counter electrode, a current flows that can be amperometrically detected and that is proportional to the number of hybridization events.

[087] This principle of amperometric detection will be explained in greater detail using the example of glucose oxidase to represent a photoinducibly redox-active moiety or a redox-active moiety. Glucose oxidase is a redox-active enzyme consisting of apoprotein and one cofactor (flavin adenine dinucleotide). The probe oligonucleotide having one end covalently attached to the electrode can be functionalized at the other, free end with the complete glucose oxidase enzymatic moiety, for example by covalently attaching the flavin adenine dinucleotide (FAD)

cofactor of the enzyme to the probe oligonucleotide and subsequently reconstituting it with the glucose oxidase apoprotein (GOx). The resultant surface hybrid having the general structure elec-spacer-ss-oligo-spacer-FAD(GOx) exhibits little or no conductivity between the electrode and the FAD. In the case of hybridization with the "ss-oligo"-complementary target oligonucleotide, the conductivity is significantly increased. Upon adding the glucose substrate to the target oligonucleotide solution, the FAD of the glucose oxidase (FAD(GOx)) is reduced to $FADH_2$ of the glucose oxidase ($FADH_2$(GOx)), glucose being oxidized to gluconic acid. If a suitable external potential is then applied to the electrode such that electrons from $FADH_2$(GOx) are given up to the electrode via the hybridized oligonucleotide, and $FADH_2$(GOx) is thus reoxidized to FAD(GOx) (but neither glucose nor gluconic acid can be electrooxidized or electroreduced at this potential), a current will flow in the elec-spacer-ds-oligo-spacer-FAD(GOx) system as long as FAD(GOx) is reduced by free glucose, i.e. until all of the glucose is consumed or, in the event that a potential at which gluconic acid can be reduced to glucose is applied to the counter electrode, as long as gluconic acid is reduced on the counter electrode. This current can be detected amperometrically and is proportional to the number of hybridization events.

[088] The photoinducibly or chemically-inducibly redox-active moieties of relevance to the present invention possess, however, instead of one electron donor or electron acceptor, at least one electron donor and at least one electron acceptor.

[089] In the case of a chemically-inducibly redox-active moiety, within the meaning of the present invention, at least one charge transfer step intervenes between electron donor(s) and electron acceptor(s). The free redox-active substance that reduces D (or oxidizes A) and thus initiates an electron transfer from D^- to A to form A^- (or an electron transfer from D to A^+ to form D^+), makes it possible to set the electrode to a potential at which A^- (or D^+), but not A (or D) can be oxidized (or reduced). This has the advantage that the electrode possesses a potential at which the direct reaction of the free redox-active substance with the electrode can be significantly suppressed, and primarily electron transfers between the redox-active moiety and the electrode can be detected.

[090] If the redox-active moiety is a photoinducibly redox-active moiety, the redox activity of the moiety is triggered only by light of a specific or any given wavelength. According to the present invention, this property is used to advantage in that electrochemical detection is triggered only by radiating light onto the surface hybrid having the general structure elec-spacer-ds-oligo-spacer-moiety (surface hybrid with hybridized target) and is maintained, at most, as long as light irradiation continues. Thus, particularly in the case of amperometric detection, if a photoinducibly redox-active moiety is used, under certain external conditions, (rather long-lasting) current will flow only if light is radiated onto the surface hybrid. Such external conditions are for example the presence of a reducing agent (or oxidizing agent) suitable for reducing (or oxidizing) a photoinductively-formed oxidized donor D^+ (or reduced acceptor A^-) of the photoinducibly redox-active moiety, and applying to the electrode a potential at which a photoinductively-formed reduced acceptor A^- (or oxidized donor D^+) of the photoinducibly redox-active moiety can be oxidized (or reduced), but the non-reduced acceptor A (or the non-oxidized donor D) cannot be oxidized (or reduced). In the section "Manner of Executing the Invention," this is explained in greater detail using various examples of an elec-spacer-ss-oligo-spacer-moiety having a photoinducibly redox-active moiety. In this way, detection using a photoinducibly redox-active moiety can be spatially limited to a certain test site or group of test sites of the oligomer chip by restricting the light to this test site or group of test sites. According to the present invention, various test sites (nucleic acid oligomer combinations) of an oligomer chip can thus be applied to a shared, continuous, electrically-conductive surface. A particular test site or group of test sites can be addressed and amperometrically detected simply by applying a suitable external potential to the (entire) surface if precisely this test site or group of test sites is irradiated with light. The various test sites thus need not be applied to individual (micro-) electrodes that are electrically isolated from one another and individually controllable for applying a potential and reading out the current. Moreover, if surface hybrids having the general structure elec-spacer-ss-oligo-spacer-moiety are used with a photoinducibly redox-active moiety and amperometric detection, the read-out process for detecting individual sequence-specific hybridization events on the oligomer chip can be optimized by first reading out the test sites by

roughly scanning them with appropriately focused light and then successively increasing the resolution capacity in the grids having hybridization events, so for example, for an octamer chip having 65,536 test sites, e.g. 64 groups of 1024 test sites each are read out, then the test site groups that are shown by amperometric measurements to exhibit hybridization events can be tested e.g. in 32 groups of 32 test sites each, and thereafter, in the test site groups that again exhibit hybridization events, the test sites are assayed individually. In this way, the individual hybridization events can be quickly assigned to specific probe oligomers with little experimental outlay.

[091] BRIEF DESCRIPTION OF THE DRAWINGS

[092] The invention will be explained in greater detail below by reference to exemplary embodiments in association with the drawings, wherein:

[093] Fig. 1 Shows a schematic diagram of oligonucleotide sequencing by hybridization on a chip;

[094] Fig. 2 Shows various reaction sequences for producing the surface hybrid elec-spacer-ss-oligo-spacer-UQ(RC). The photoinducibly redox-active moiety in this surface hybrid is the reaction center (RC) of the photosynthesizing bacteria *Rhodobacter sphaeroides*. This photoinducibly redox-active protein consists of apoprotein and cofactors. The RC, via its cofactor ubiquinone-50 (UQ) in what is known as the Q_A protein binding pocket, is covalently joined via a spacer with the oligonucleotide;

[095] Fig. 3 Shows a schematic diagram of the photoinduced amperometric measurement method using the example of the surface hybrid elec-spacer-ss-oligo-spacer-UQ(RC) in Figure 2 (hν: irradiation with light; P: primary donor of the RC; UQ: ubiquinone-50 electron acceptor in the Q_A protein binding pocket of the RC; Red/Ox: reduced or oxidized form of the free redox-active substance added to the target solution, e.g. cyt c_2^{2+}, sodium ascorbate or $Fe(CN)_6^{2+}$, which can rereduce the oxidized form P^+ to its original neutral state P; E_{Ox}: potential of the electrode at which UQ^- is oxidized to UQ via electron transfer to the electrode; "hν on": beginning of light irradiation; "hν off": end of light irradiation);

[096] Fig. 4 Shows a detailed schematic diagram of the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-Q-ZnBChl of Figure 3 having gold as the solid support material, mercaptoethanol as the spacer (-S-CH_2CH_2- spacer) between the electrode and the oligonucleotide, and -CH_2-CH=CH-CO-NH-CH_2-CH_2-NH- as the spacer between the electron acceptor PQQ and the oligonucleotide, as well as a diagram of the sequence of the photoinduced electron transfer steps. The apoprotein of the RC is indicated only as a shell (solid line) (cf. Structure 1). The 12-bp probe oligonucleotide of the exemplary sequence 5'-TAGTCGGAAGCA-3' in the hybridized state is shown in detail;

[097] Fig. 5 Shows a schematic diagram of the photoinduced amperometric measurement method using the example of the surface hybrid elec-spacer-ss-oligo-spacer-Q-ZnBChl (hν: irradiation with light; ZnBChl: electron-donor molecule zinc-bacteriochlorophyll; Q: the electron-acceptor molecule quinone, e.g. modified anthraquinone or PQQ; Red/Ox: reduced or oxidized form of the free redox-active substance added to the target solution, e.g. $Fe(CN)_6^{2+}$, which can rereduce the oxidized form of the electron donor $ZnBChl^+$ to its original neutral state ZnBChl; E_{Ox}: electrode potential at which Q^- is oxidized to Q by giving up an electron to the electrode; "hν on": beginning of light irradiation; "hν off": end of light irradiation);

[098] Fig. 6 Shows a detailed schematic diagram of the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-Q-ZnBChl of Figure 5 having gold as the solid support material, mercaptoethanol as the spacer (-S-CH_2CH_2- spacer) between the electrode and the oligonucleotide, and -CH_2-CH=CH-CO-NH-CH_2-CH_2-NH- as the spacer between the electron acceptor PQQ and the oligonucleotide, as well as a diagram of the sequence of the photoinduced electron transfer steps. The 12-bp probe oligonucleotide of the exemplary sequence 5'-TAGTCGGAAGCA-3' in the hybridized state is shown in detail.

[099] MANNER OF EXECUTING THE INVENTION

[100] A formation unit of an exemplary test site with hybridized target, Au-S$(CH_2)_2$-ds-oligo-spacer-UQ(RC) having the general structure elec-spacer-ds-oligo-spacer-moiety, is illustrated in Figure 4. In the context of the present invention, "formation unit" is understood to mean the smallest repeating unit of a test site. In the example in Figure 4, the surface is a gold electrode. The link between the gold electrode and the probe oligonucleotide was formed with the linker (HO-$(CH_2)_2$-S$)_2$, which was esterified with the terminal phosphate group at the 3'-end to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH and, following homolytic cleavage of the S-S bond at the gold surface, produced one Au-S bond each, with which 2-hydroxy-mercaptoethanol and mercaptoethanol-bridged oligonucleotide was coadsorbed on the surface. The photoinducibly redox-active moiety in the example in Figure 4 is the reaction center (RC) of the photosynthesizing bacteria *Rhodobacter sphaeroides*, a photoinducibly redox-active protein consisting of apoprotein and cofactors. In the application example, the RC, via its cofactor ubiquinone-50 (UQ) in what is known as the Q_A binding pocket of the RC, is covalently joined with the oligonucleotide, where free UQ was first provided with a reactive carboxylic-acid group (see Example 1), then covalently attached to the probe oligonucleotide via this carboxylic-acid group (amidation and dehydration of the terminal amino function of the -CH=CH-CO-NH-CH_2-CH_2-NH_2 linker attached at the C-5 position of the 5' thymine),

and finally, the remaining RC (apoprotein with all cofactors except UQ) was reconstituted to UQ.

[101] As already mentioned above, the modification of the probe oligonucleotides may take place with the complete redox-active moiety or with a component thereof, either before or after the probe oligonucleotide is bound to the conductive surface. The various combination possibilities of the individual steps, which lead in effect to the same test site formation unit, will be illustrated below with the aid of Figure 2 using the example of the surface hybrid Au-S$(CH_2)_2$-ss-oligo-spacer-UQ(RC) or its more general form elec-spacer-ss-oligo-spacer-UQ(RC).

[102] The reaction center can be freed of the two ubiquinone cofactors in the Q_A or Q_B binding pocket by simple manipulation (Gunner, M.R., Robertson, D.E., Dutton, P.L., 1986, Journal of Physical Chemistry, Vol. 90, pp. 3783-3795), thus yielding ubiquinone separated from the remaining RC (apoprotein including all cofactors except ubiquinone in the Q_A or Q_B binding pocket). The probe oligonucleotide has (identical or differing) reactive groups attached, via a (any) spacer, near each of its ends. In a reaction sequence "1," in the presence of a monofunctional linker (according to points a) - c) and 2.) in the section "Binding an Oligonucleotide to the Conductive Surface"), the probe oligonucleotide thus modified may be covalently attached to the electrode together with the monofunctional linker, making sure that sufficient monofunctional linker of suitable chain length is added to provide sufficient space between the individual probe oligonucleotides to permit hybridization with the target oligonucleotide and to permit the attachment of the redox-active moiety. Thereafter, UQ that was previously provided with a suitable reactive coupling group is attached to the free spacer-bridged reactive group of the probe oligonucleotide. The attachment takes place as described under a) or b) in the section "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer." In the last step of this reaction sequence "1," the remaining RC (apoprotein with all cofactors except UQ) is reconstituted to UQ. In a variation hereof (reaction sequence "2"), the modified (with spacer and reactive groups) probe oligonucleotide can first be covalently bound to the

electrode without a free monofunctional linker (spacer), causing a flat attachment of the oligonucleotide. Thereafter, the free monofunctional linker (spacer) is covalently bound to the electrode. A further possibility (reaction sequence "3") is to modify the modified (with spacer and reactive groups) probe oligonucleotide first with UQ, then covalently attach it to the electrode in the presence of a free monofunctional linker (spacer), and thereafter reconstitute it with the remaining RC. Finally, in a reaction sequence "4," the modified (with spacer and reactive groups) probe oligonucleotide can first be modified with UQ so as then to reconstitute it with the remaining RC and, thereafter, to covalently bind it to the electrode. In the event that, as in the case of the RC, the redox-active moiety has a significantly greater diameter than the hybridized ds-oligonucleotide (greater than 30 Å), the covalent attachment of a suitable free monofunctional linker (spacer) to the electrode can be dispensed with; otherwise, the attachment of the structure -spacer-ss-oligo-spacer-UQ(RC) to the electrode occurs in the presence of a suitable free monofunctional linker.

[103] In the example in Figure 2, the RC, via its cofactor ubiquinone-50 (UQ) in what is known as the Q_A protein binding pocket of the RC, is covalently joined with the oligonucleotide. Alternatively, instead of the UQ cofactor in the Q_A binding pocket, another cofactor of the RC or the apoprotein can also be covalently attached to the probe oligonucleotide, any combinations of the reaction sequences "1," "2," "3," or "4" in Figure 2 may be applied, as long as they yield the same end product (cf. Figure 2), and, in any reaction steps, the probe oligonucleotide hybridized with complementary, unmodified (target) oligonucleotide may be used in place of the single-strand probe oligonucleotides. The probe oligonucleotide can also be attached directly, in other words not bridged via a spacer, to both the electrode and the redox-active moiety, as described under c) in the section "Binding a Nucleic Acid Oligomer to the Conductive Surface" or under a) in the section "Binding a Redox-Active Moiety to a Nucleic Acid Oligomer."

[104] The electrical communication between the conductive surface and the redox-active moiety bridged via a single-strand oligonucleotide in the general structure elec-spacer-ss-oligo-spacer-moiety is weak or

nonexistent. If hybridization occurs between the probe and the target, treating the test site(s) with an oligonucleotide solution to be examined leads to increased conductivity between the surface and the redox-active moiety bridged via a double-strand oligonucleotide. For the formation unit of the test site Au-S$(CH_2)_2$-ds-oligo-spacer-UQ(RC) (with 12-bp probe oligonucleotides) used as an example, this is shown schematically in Figure 3 using amperometric measurements.

[105] The cofactor P, also called the primary donor, is electronically excited by irradiating the RC with light of a suitable wavelength, causing photoinduced charge separation within the cofactors of the RC, an electron being transferred from the excited primary donor P* to the UQ in the Q_A binding pocket. If a suitable potential is applied to the electrode to transfer an electron from the reduced ubiquinone (UQ^-) to the electrode, current still will not flow in the case of the probe oligonucleotide not hybridized with the target oligonucleotide, since the conductivity of the ss-oligonucleotide in Au-S$(CH_2)_2$-ss-oligo-spacer-UQ(RC) is very slight or nonexistent. In the hybridized state (Au-S$(CH_2)_2$-ds-oligo-spacer-UQ(RC)), however, conductivity is high, an electron can be transferred from UQ^- to the electrode (forming UQ), and, in the presence of a suitable redox-active substance that reduces P^+ to P, the circuit is closed and further light absorption by the RC begins the cycle anew. This manifests itself amperometrically in a distinct flow of current between the electrode and the photoinducibly redox-active moiety (Figure 3). It is thus possible to detect the sequence-specific hybridization of the target with the probe oligonucleotides by amperometry using photoinduction. The individual electron transfer steps that are triggered in the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-UQ(RC) by light irradiation and in the presence of a suitable redox-active substance that reduces P^+ to P are illustrated in Figure attachment of the RC to the probe oligonucleotide near the primary donor), the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-UQ(RC) can, of course, also be reversed, such that, after light irradiation, P^+ is reduced by the electrode and Q^- is oxidized by a suitable oxidizing agent.

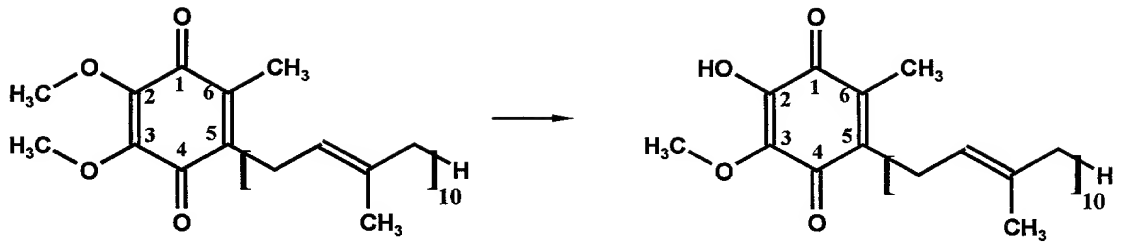
[106] A further test site, Au-S(CH_2)$_2$-ss-oligo-spacer-Q-ZnBChl, having the general structure elec-spacer-ss-oligo-spacer-moiety, is illustrated in Figure 5. By irradiating ZnBChl with light of a suitable wavelength, ZnBChl is electronically excited and photoinduced charge separation occurs, an electron being transferred from the excited ZnBChl* to the quinone Q. If a suitable potential is applied to the electrode to transfer to the electrode an electron from the quinone (Q^-) thus reduced, current still will not flow in the case of the probe oligonucleotide not hybridized with the target oligonucleotide, since the conductivity of the ss-oligonucleotide in Au-S(CH_2)$_2$-ss-oligo-spacer-Q-ZnBChl is very slight or nonexistent. In the hybridized state Au-S(CH_2)$_2$-ds-oligo-spacer-Q-ZnBChl, however, conductivity is high, an electron can be transferred from Q^- to the electrode (forming Q) and, in the presence of a suitable redox-active substance that reduces $ZnBChl^+$ to ZnBChl, the circuit is closed and further light absorption by ZnBChl begins the cycle anew. This manifests itself amperometrically in a distinct flow of current between the electrode and the photoinducibly redox-active moiety (Figure 5). The sequence-specific hybridization of the target with the probe oligonucleotides can thus be detected by amperometry using photoinduction. Given suitable external conditions and a suitable attachment (e.g. Au-S(CH_2)$_2$-ds-oligo-spacer-ZnBChl-Q), the surface hybrid Au-S(CH_2)$_2$-ds-oligo-spacer-Q-ZnBChl can, of course, also be reversed, such that, after light irradiation, $ZnBChl^+$ is reduced by the electrode and Q^- is oxidized by a suitable oxidizing agent.

[107] Since the redox activity of the photoinducibly redox-active moiety – even at the appropriate electrode potential – is triggered only by irradiation with light of a suitable wavelength and is maintained, at most, as long as the light irradiation continues, this condition can be used to advantage, according to the present invention, in that a particular test site or group of test sites of an oligomer chip is spatially resolved by restricting the light to this test site or group of test sites. The advantage of doing so, according to the present invention, is that the various test sites (nucleic acid oligomer combinations) of an oligomer chip can be applied to a shared, continuous, electrically-conductive surface and a specific test site or a specific group of test sites can be addressed and amperometrically detected simply by

applying a suitable external potential to the (entire) surface if specifically this test site or group of test sites alone is irradiated with light. The various test sites thus need not be applied to individual (micro-) electrodes that are electrically isolated from one another and individually controllable for potential application and current selection.

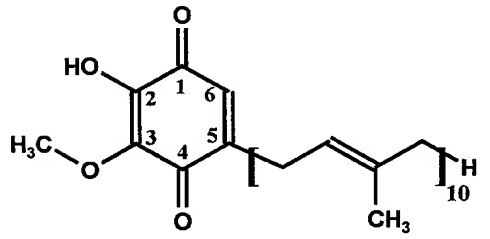
[108] In addition, defective base pairings (base-pair mismatches) can be recognized by an altered cyclic voltammetric characteristic. A mismatch manifests itself in a greater potential difference between the current maximums of electroreduction and electroreoxidation (reversal of electroreduction when the potential feed direction is reversed) or electrooxidation and electrorereduction in a cyclic voltammetrically reversible electron transfer between the electrically-conducting surface and the photoinducibly redox-active moiety. This fact has an advantageous effect most importantly on amperometric detection because there, the current can be tested at a potential at which the perfectly hybridizing oligonucleotide target supplies significant current, but the defectively paired oligonucleotide target does not.

[109] ***Example 1:*** *Modification of ubiquinone-50 with a spacer-bridged reactive carboxylic-acid group.* The 2-methoxy group of ubiquinone-50 (UQ-50) is modified to a 2-hydroxy group by ether cleavage with HBr, a standard method (alternatively, 2-OH-UQ-50 may be produced after the method of H. W. Moore, and K. Folkers, *Journal of the American Chemical Society*, 1966, 88, pp. 564-570 or of G. Daves et al., *Journal of the American Chemical Society*, 1968, 90, pp. 5587-5593). Thereafter, in a standard method using an equimolar amount of Cl-CH_2-CH_2-CO_2H, 2-OH-UQ-50 is converted to 2-(CH_2-CH_2-CO_2H)-UQ-50 and chromatographically purified. Alternatively, in a standard method, 5-OH-6-alkyl-1,4-benzoquinone analogs of UQ-50 (produced after Catlin et al., Journal of the American Chemical Society, 1968, 90, pp. 3572-3574) may be modified to 5-(CH_2-CH_2-CO_2H)-UQ-50-analogs using an equimolar amount of Cl-CH_2-CH_2-CO_2H.

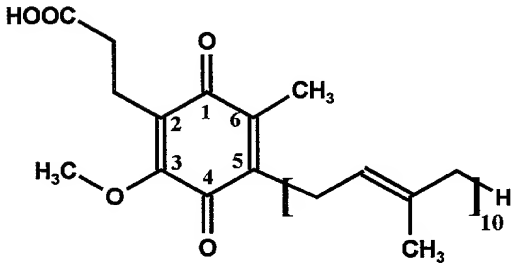


UQ-50

2-OH-UQ-50



2-OH-UQ-50



2-(CH_2-CH_2-CO_2H)-UQ-50

[110] ***Example 2:*** *Producing the oligonucleotide electrode Au-S(CH_2)$_2$-ss-oligo-spacerUQ(RC).* Au-S(CH_2)$_2$-ss-oligo-spacer-UQ(RC) is produced in 4 steps, namely producing the conductive surface, derivatizing the surface with the oligonucleotide probe in the presence of a suitable monofunctional linker (incubation step), covalently attaching the modified ubiquinone-50 (redox step) and reconstituting the remaining RC (reconstitution step).

[111] An approx. 100 nm thin gold film on mica (muscovite lamina) forms the support material for the covalent attachment of the double-strand oligonucleotides. For this purpose, freshly cleaved mica was purified with an argon-ion plasma in an electrical discharge chamber and gold (99.99%) was applied by electrical discharge in a layer thickness of approx. 100 nm. Thereafter, the gold film was freed of surface impurities (oxidation of

organic accumulations) with 30% H_2O_2, / 70% H_2SO_4 and immersed in ethanol for approx. 20 minutes to dispel any oxygen adsorbed on the surface. After rinsing the surface with bidistilled water, a previously prepared $1x10^{-4}$ molar solution of the (modified) double-strand oligonucleotide is applied on the horizontally mounted surface, such that the entire gold surface is wetted (incubation step, see also below).

[112] For incubation, a doubly modified 12-bp single-strand oligonucleotide having the sequence 5'-TAGTCGGAAGCA-3' was used, which is esterified with $(HO-(CH_2)_2-S)_2$ at the phosphate group of the 3'-end to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH. At the 5'-end, the terminal thymine base of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH_2-CH_2-NH_2. Approximately 10^{-4} to 10^{-1} molar 2-hydroxy-mercaptoethanol (or another thiol or disulfide linker having a suitable chain length) was added to a $2x10^{-4}$ molar solution of this oligonucleotide in HEPES buffer (0.1 molar in water, pH 7.5 with 0.7 molar addition of TEATFB, see abbreviations) and the gold surface of a test site was completely wetted and incubated for 2-24 hours. During this reaction time, the disulfide spacer P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH of the oligonucleotide is homolytically cleaved. In this process, the spacer forms a covalent Au-S bond with Au atoms of the surface, thus causing a 1:1 coadsorption of the ss-oligonucleotide and the cleaved 2-hydroxy-mercaptoethanol. The free 2-hydroxy-mercaptoethanol that is also present in the incubation solution is likewise coadsorbed by forming an Au-S bond (incubation step).

[113] The gold electrode thus modified with a monolayer comprising ss-oligonucleotide and 2-hydroxy-mercaptoethanol was washed with bidistilled water and subsequently wetted with a solution of $3x10^{-3}$ molar quinone 2-(CH_2-CH_2-CO_2H)-UQ-50, 10^{-2} molar EDC, and 10^{-2} molar sulfo-NHS in HEPES buffer (0.1 molar (in water, pH = 7.5). After a reaction time of approx. 1 - 4 hours, the -CH=CH-CO-NH-CH_2-CH_2-NH_2 spacer and the 2-(CH_2-CH_2-CO_2H)-UQ-50 form a covalent bond (amidation between the amino group of the spacer and the C-2-acid function of 2-(CH_2-CH_2-CO_2H)-UQ-50, redox step).

[114] Thereafter, the gold electrode thus modified was washed with bidistilled water and incubated for approx. 12 hours with a solution of approx. $5x10^{-5}$ molar ubiquinone-50-free RC in 10 mM Tris, pH = 8, with 0.7 molar addition of TEATFB at approx. 4°C, to reconstitute the remaining RC to the UQ-50 bound to the oligonucleotide (reconstitution step).

[115] Alternatively, to covalently attach 2-(CH_2-CH_2-CO_2H)-UQ-50 to the probe oligonucleotide, under the same conditions, a 5-(CH_2-CH_2-CO_2H)-UQ-50-analog (Example 1) or another quinone of the Formula 1 – 8 having a reactive carboxylic acid may also be used, since ubiquinone-50-free RC can also be reconstituted to these.

[116] *Example 3: Producing the oligonucleotide electrode Au-S(CH_2)$_2$-ss-oligo-spacer-Q-ZnBChl.* Au-S(CH_2)$_2$-ss-oligo-spacer-Q-ZnBChl is produced in 5 steps, namely producing the conductive surface, derivatizing the surface with the probe oligonucleotide (hybridized with a complementary strand) in the presence of a suitable monofunctional linker (incubation step), covalently attaching the electron acceptor (acceptor step), covalently attaching the electron donor (donor step), and thermally dehybridizing the double-strand oligonucleotide (dehybridization step).

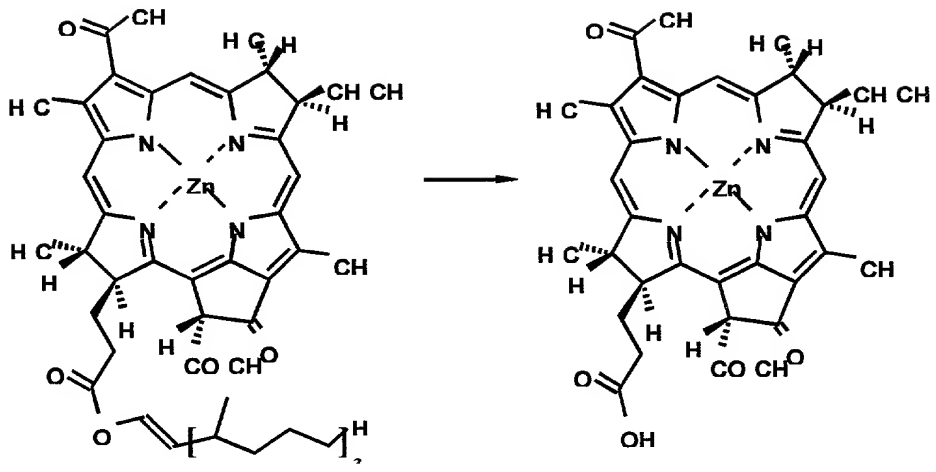
[117] The support material for the covalent attachment of the double-strand oligonucleotides, an approx. 100 nm thin gold film on mica (muscovite lamina), was produced as described in Example 1.

[118] For incubation, a doubly modified 12-bp single-strand oligonucleotide having the sequence 5'-TAGTCGGAAGCA-3' was used, which is esterified with (HO-(CH_2)$_2$-S)$_2$ at the phosphate group of the 3'-end to form P-O-(CH_2)$_2$-S-S-(CH_2)$_2$-OH. At the 5'-end, the terminal thymine base of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH_2-CH_2-NH_2. A $2x10^{-4}$ molar solution of this oligonucleotide in the hybridization buffer (10 mM Tris, 1 mM EDTA, pH 7.5 with 0.7 molar addition of TEATFB, see abbreviations) was hybridized with a $2x10^{-4}$ molar solution of the (unmodified) complementary strand in the hybridization buffer at room temperature for approx. 2 hours (hybridization step). After hybridization, approx. 10^{-4} to 10^{-1} molar 2-hydroxy-mercaptoethanol (or another thiol or disulfide linker having a suitable chain length) was added to the now $1x10^{-4}$ molar double-strand

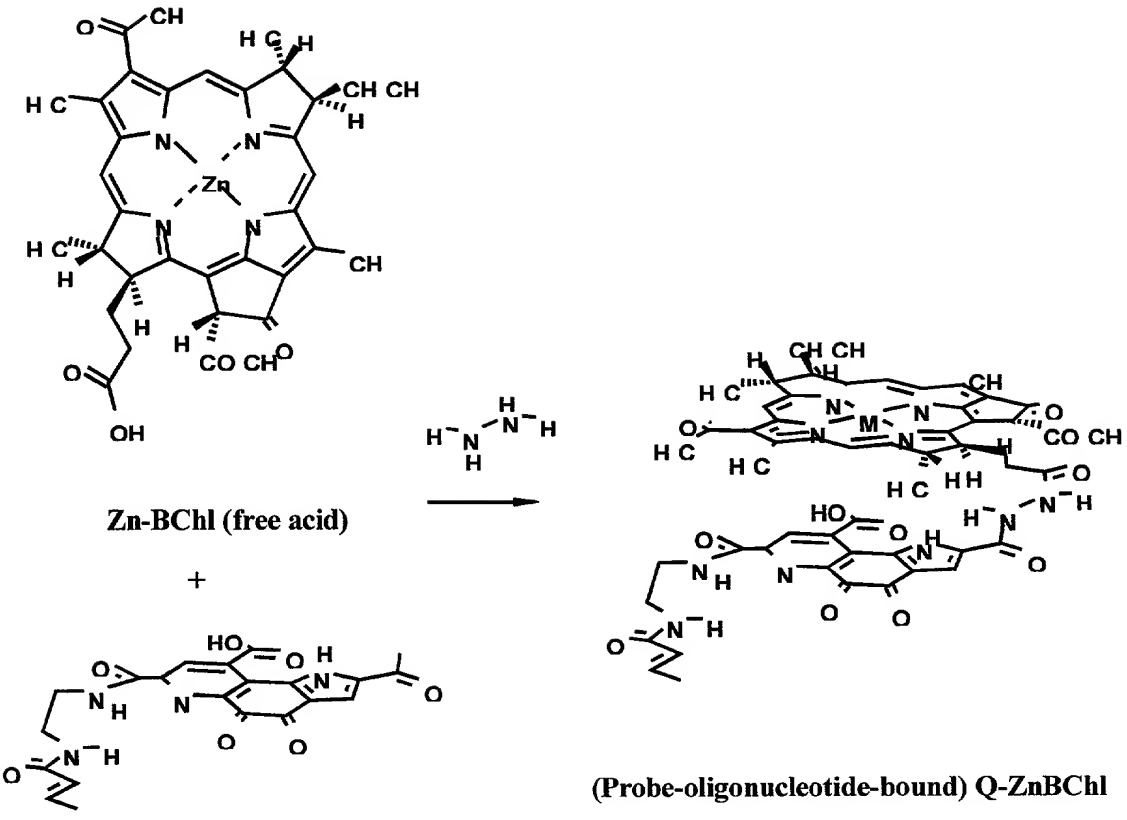
oligonucleotide solution and the gold surface of a test site was completely wetted and incubated for 2 - 24 hours. During this reaction time, the disulfide spacer P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH of the oligonucleotide is homolytically cleaved. In this process, the spacer forms a covalent Au-S bond with Au atoms of the surface, thus causing a 1:1 coadsorption of the ds-oligonucleotide and the cleaved 2-hydroxy-mercaptoethanol. The free 2-hydroxy-mercaptoethanol that is also present in the incubation solution is likewise coadsorbed by forming an Au-S bond (incubation step).

[119] The gold electrode thus modified with a monolayer comprising ds-oligonucleotide and 2-hydroxy-mercaptoethanol was washed with bidistilled water and subsequently wetted with a solution of $3x10^{-3}$ molar quinone PQQ, 10^{-2} molar EDC, and 10^{-2} molar sulfo-NHS in HEPES buffer. After a reaction time of approx. 1 - 4 hours, the -CH=CH-CO-NH-CH_2-CH_2-NH_2 spacer and the PQQ form a covalent bond (amidation between the amino group of the spacer and the C-7-carboxylic-acid function of the PQQ, acceptor step).

[120] Thereafter, the gold electrode thus modified was washed with bidistilled water and wetted with an aqueous solution of $3x10^{-3}$ molar donor ZnBChl (free acid), $1.5x10^{-1}$ molar EDC, $2.5x10^{-3}$ molar hydrazine monohydrate (NH_2-NH_2xH_2O), and $1x10^{-1}$ molar imidazole. After a reaction time of approx. 16 hours at 23°C, the C-1-carboxylic-acid function of the PQQ bound to the oligonucleotide binds via hydrazine to the free carboxylic-acid group of the ZnBChl (amidation between the amino groups of the hydrazine and the C-1-carboxylic-acid group of the PQQ or the free carboxylic-acid group of the ZnBChl, donor step). Thereafter, the double-strands were thermally dehybridized at temperatures of T > 40°C and rinsed again with bidistilled water (dehybridization step). The ZnBChl (free acid) is produced from Zn-BChl (produced after Hartwich et al., Journal of the American Chemical Society, 1998, 120, pp. 3684-3693) by incubation with trifluoroacetic acid.



Zn-BChl **Zn-BChl (free acid)**


Zn-BChl (free acid)
+
(Probe-oligonucleotide-bound) Q
(Probe-oligonucleotide-bound) Q-ZnBChl

[121] Alternatively, for example, ZnBChl (free acid) can also be bound to the 3-OH group of the 5'-terminal sugar of the probe oligonucleotide by esterification according to standard methods, or the previously covalently-joined electron-donor/electron-acceptor complex is attached to the probe oligonucleotide, as described in the donor step, via a free carboxylic-acid group, for example of the donor. In place of PQQ, under the same reaction conditions, anthraquinone-2,6-disulfonic acid disodium salt can also be used in the acceptor step. If PNA oligonucleotide is used with, for example, -NH-$(CH_2)_2$-N($COCH_2$-base)-CH_2CO- as the oligonucleotide building block, there exists an alternative possibility for attaching the ZnBChl-PQQ moiety to the nucleic acid oligomer (PNA oligonucleotide) according to d) in the section "Binding a Photoinducibly Redox-Active Moiety to a Nucleic Acid Oligomer." In this case, during PNA oligonucleotide synthesis, instead of the N-terminal base in the standard PNA synthesis reaction, PQQ is attached via its pyrrole nitrogen. Thereafter, in a manner similar to that described in the donor step, Zn-BChl is bound to the amino end of the peptide backbone by incubating the PNA oligonucleotide modified with PQQ with $3x10^{-3}$ molar ZnBChl (free acid), $1.5x10^{-1}$ molar EDC 10^{-2} and $2x10^{-1}$ molar sulfo-NHS in HEPES buffer (amidation between the amino group of the backbone and the carboxylic-acid group of the Zn-BChl (free acid)).

Claims

1. A nucleic acid oligomer modified by covalently attaching a redox-active moiety, characterized in that the redox-active moiety comprises one or more electron-donor molecules and one or more electron-acceptor molecules.

2. The modified nucleic acid oligomer according to claim 1, characterized in that the redox-active moiety comprises at least one redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex, at least two of the electron-donor molecule(s) and/or electron-acceptor molecule(s) of the redox-active moiety being joined with one another via one or more bonds.

3. The modified nucleic acid oligomer according to claim 2, characterized in that at least two of the electron-donor molecule(s) and/or electron-acceptor molecule(s) of the redox-active moiety are joined with one another via one or more covalent bonds.

4. The modified nucleic acid oligomer according to claim 1, characterized in that the redox-active moiety comprises at least one redox-active, linked, at least bimolecular electron-donor/electron-acceptor complex, at least two of the electron-donor molecule(s) and/or electron-acceptor molecule(s) being covalently joined via one or more branched or linear molecular moieties of any composition and chain length.

5. The modified nucleic acid oligomer according to claim 4, wherein the branched or linear molecular moieties have a chain length of 1 – 20 atoms, especially 1 – 14 atoms.

6. The modified nucleic acid oligomer according to one of the preceding claims, characterized in that the redox-active moiety comprising one

or more electron-donor molecules and one or more electron-acceptor molecules additionally comprises one or more macromolecules.

7. The modified nucleic acid oligomer according to one of the preceding claims, wherein the redox-active moiety is the native or modified reaction center of photosynthesizing organisms, especially the native or modified reaction center of photosynthesizing bacteria.

8. The modified nucleic acid oligomer according to one of claims 1 through 6, characterized in that one or more of the electron-donor and/or electron-acceptor molecule(s) are pigments, especially flavins, (metallo)porphyrins, (metallo)chlorophylls, or (metallo)bacteriochlorophylls, or derivatives thereof.

9. The modified nucleic acid oligomer according to one of claims 1 through 6, characterized in that one or more of the electron-donor and/or electron-acceptor molecule(s) are nicotinamides or quinones, especially pyrrolo-quinoline quinones (PQQ), 1,2-benzoquinones, 1,4-benzoquinones, 1,2-naphthoquinones, 1,4-naphthoquinones or 9,10-anthraquinones, or derivatives thereof.

10. The modified nucleic acid oligomer according to one of claims 1 through 6, characterized in that one or more of the electron-donor and/or electron-acceptor molecule(s) are charge transfer complexes.

11. The modified nucleic acid oligomer according to claim 10, wherein the charge transfer complex is a transition metal complex, especially a Ru(II), Cr(III), Fe(II), Os(II), or Co(II) complex.

12. The modified nucleic acid oligomer according to one of the preceding claims, wherein the modified nucleic acid oligomer can sequence-specifically bind single-strand DNA, RNA, and/or PNA.

13. The modified nucleic acid oligomer according to claim 12, wherein the modified nucleic acid oligomer is a deoxyribonucleic acid

oligomer, a ribonucleic acid oligomer, a peptide nucleic acid oligomer, or a nucleic acid oligomer having a structurally analogous backbone.

14. The modified nucleic acid oligomer according to one of the preceding claims, wherein, alternatively, the redox-active moiety is covalently bound to one of the phosphoric-acid, carboxylic-acid, or amine groups, or to a sugar, especially to a sugar-hydroxyl group, of the nucleic acid oligomer backbone.

15. The modified nucleic acid oligomer according to one of claims 1 through 13, wherein, alternatively, the redox-active moiety is covalently attached to a thiol, hydroxyl, carboxylic-acid, or amine group of a modified base of the nucleic acid oligomer.

16. The modified nucleic acid oligomer according to claim 15, characterized in that the reactive thiol, hydroxyl, carboxylic-acid, or amine group of the base is covalently bound to the base via a branched or linear molecular moiety having any composition and chain length, the shortest continuous link between the thiol, hydroxyl, carboxylic-acid, or amine group and the base being a branched or linear molecular moiety having a chain length of 1 – 20 atoms, and especially of 1 – 14 atoms.

17. The modified nucleic acid oligomer according to one of claims 14 through 16, wherein the redox-active moiety is attached to an end of the nucleic acid oligomer backbone or to a terminal, modified base.

18. The modified nucleic acid oligomer according to one of the preceding claims, characterized in that the redox-active moiety is a photoinducibly redox-active moiety.

19. The modified nucleic acid oligomer according to one of claims 1 through 17, characterized in that the redox-active moiety is a chemically-inducibly redox-active moiety.

20. The modified nucleic acid oligomer according to one of the preceding claims, characterized in that multiple redox-active moieties are attached to the nucleic acid oligomer.

21. A method of producing a modified nucleic acid oligomer as defined in one of the preceding claims, wherein a redox-active moiety is covalently attached to a nucleic acid oligomer.

22. The method of producing a modified nucleic acid oligomer according to claim 21, wherein the redox-active moiety is attached to a nucleic acid oligomer by covalently attaching one or more electron-donor molecule(s).

23. The method of producing a modified nucleic acid oligomer according to claim 21, wherein the redox-active moiety is attached to a nucleic acid oligomer by covalently attaching one or more electron-acceptor molecule(s).

24. The method of producing a modified nucleic acid oligomer according to claim 21, wherein the redox-active moiety is attached to a nucleic acid oligomer by covalently attaching one or more macromolecules or by covalently attaching one or more proteins.

25. The method of producing a modified nucleic acid oligomer according to claims 22 through 24, wherein the redox-active moiety is completed by adding one or more electron-acceptor molecule(s), one or more electron-donor molecule(s), one or more macromolecules, and/or one or more proteins.

26. The method of producing a modified nucleic acid oligomer according to one of claims 21 through 25, wherein, alternatively, the nucleic acid oligomer is bound to the redox-active moiety by one or more amidations with amine or acid groups of the redox-active moiety, by one or more esterifications with alcohol or acid groups of the redox-active moiety, by thioester formation with thioalcohol or acid groups of the redox-active moiety,

or by condensation of one or more amine groups of the nucleic acid oligomer with aldehyde groups of the redox-active moiety and subsequent reduction of the resultant carbon-nitrogen double bond.

27. The method of producing a modified nucleic acid oligomer according to one of claims 21 through 26, wherein one or more branched or linear molecular moieties of any composition and chain length are covalently attached to the redox-active moiety and the branched or linear molecular moieties possess, alternatively, a reactive amine, hydroxyl, thiol, acid, or aldehyde group for covalent attachment to a nucleic acid oligomer.

28. The method of producing a modified nucleic acid oligomer according to claim 27, wherein the shortest continuous link between the nucleic acid oligomer and the redox-active moiety is a branched or linear molecular moiety having a chain length of 1 – 20 atoms, and especially of 1 – 14 atoms.

29. A modified conductive surface, characterized in that one or more types of modified nucleic acid oligomers according to one of claims 1 through 20 are attached to a conductive surface.

30. The modified conductive surface according to claim 29, wherein the surface consists of a metal or a metal alloy, especially a metal selected from the group: platinum, palladium, gold, cadmium, mercury, nickel, zinc, carbon, silver, copper, iron, lead, aluminum, manganese, and their mixtures.

31. The modified conductive surface according to claim 29, wherein the surface consists of a semiconductor, especially a semiconductor selected from the group: carbon, silicon, germanium, and α-tin.

32. The modified conductive surface according to claim 29, wherein the surface consists of a binary compound of the elements of groups 14 and 16, a binary compound of the elements of groups 13 and 15, a binary

compound of the elements of groups 15 and 16, or a binary compound of the elements of groups 11 and 17, especially a Cu(I) halide or an Ag(I) halide.

33. The modified conductive surface according to claim 29, wherein the surface consists of a ternary compound of the elements of groups 11, 13, and 16, or a ternary compound of the elements of groups 12, 13, and 16.

34. The modified conductive surface according to claims 29 through 33, wherein the attachment of the modified nucleic acid oligomers to the conductive surface occurs covalently or by chemisorption or physisorption.

35. The modified conductive surface according to one of claims 29 through 34, wherein, alternatively, one of the phosphoric-acid, carboxylic-acid, or amine groups, or a sugar group, especially a sugar-hydroxy group of the nucleic acid oligomer backbone, is attached, covalently or by chemisorption or physisorption, to the conductive surface.

36. The modified conductive surface according to one of claims 29 through 34, characterized in that, alternatively, a thiol, hydroxyl, carboxylic-acid, or amine group of a modified base of the nucleic acid oligomer is attached, covalently or by chemisorption or physisorption, to the conductive surface.

37. The modified conductive surface according to claim 35 or 36, wherein the modified nucleic acid oligomer is bound to the conductive surface via a group at the end of the nucleic acid oligomer backbone or via a group of a terminal, modified base.

38. The modified conductive surface according to claims 29 through 37, wherein branched or linear molecular moieties of any composition and chain length are attached, covalently or by chemisorption or physisorption, to the conductive surface and the modified nucleic acid oligomers are covalently attached to these molecular moieties.

39. The modified conductive surface according to claim 38, wherein the shortest continuous link between the conductive surface and the nucleic acid oligomer is a branched or linear molecular moiety having a chain length of 1 – 20 atoms, and especially of 1 – 12 atoms.

40. The modified conductive surface according to claim 38 or 39, wherein, alternatively, the branched or linear molecular moiety is attached to a phosphoric-acid, carboxylic-acid, or an amine group, or a sugar group, especially a sugar-hydroxyl group, of the nucleic acid oligomer backbone, or a thiol, hydroxyl, carboxylic-acid, or amine group of a modified base of the nucleic acid oligomer.

41. The modified conductive surface according to claim 40, wherein the branched or linear molecular moiety is bound to a phosphoric-acid, sugar-hydroxyl, carboxylic-acid, or amine group at the end of the nucleic acid oligomer backbone or to a thiol, hydroxyl, carboxylic-acid, or amine group of a terminal, modified base.

42. The modified conductive surface according to one of claims 29 through 41, characterized in that predominantly one type of modified nucleic acid oligomer each is attached in a spatially delimited area of the conductive surface.

43. The modified conductive surface according to one of claims 29 through 41, characterized in that only one type of modified nucleic acid oligomer each is attached in a spatially delimited area of the conductive surface.

44. A method of producing a modified conductive surface as defined in claims 29 through 43, characterized in that one or more types of modified nucleic acid oligomers are applied to a conductive surface.

45. The method of producing a modified conductive surface as defined in claims 29 through 43, characterized in that one or more types of

nucleic acid oligomers are applied to a conductive surface and, thereafter, a modification of the nucleic acid oligomers is carried out using a method according to claims 21 through 28.

46. The method of producing a modified conductive surface according to claim 44 or 45, wherein the nucleic acid oligomers or the modified nucleic acid oligomers are hybridized with the respective complementary nucleic acid oligomer strand and applied to the conductive surface in the form of the double-strand hybrid.

47. The method of producing a modified conductive surface according to claim 44 or 45, wherein the nucleic acid oligomer or the modified nucleic acid oligomer is applied to the conductive surface in the presence of further chemical compounds that are likewise attached to the conductive surface.

48. A method of electrochemically detecting oligomer hybridization events, characterized in that one or more modified conductive surfaces as defined in claims 29 through 43 are brought into contact with nucleic acid oligomers and, subsequently, detection of the electrical communication between the redox-active moiety and the respective conductive surface takes place.

49. The method according to claim 48, wherein detection takes place by cyclic voltammetry, amperometry, or conductivity measurement.

50. The method of electrochemical detection according to claim 48 or 49, characterized in that electrochemical detection is initiated by photoinduced charge separation in the photoinducibly redox-active moiety attached to the conductive surface via a nucleic acid oligomer.

51. The method according to claim 50, wherein the light irradiation for photoinduced charge separation in the photoinducibly redox-active moiety attached to the conductive surface via a nucleic acid oligomer is limited to an

area of the conductive surface having one or more modified nucleic acid oligomer types.

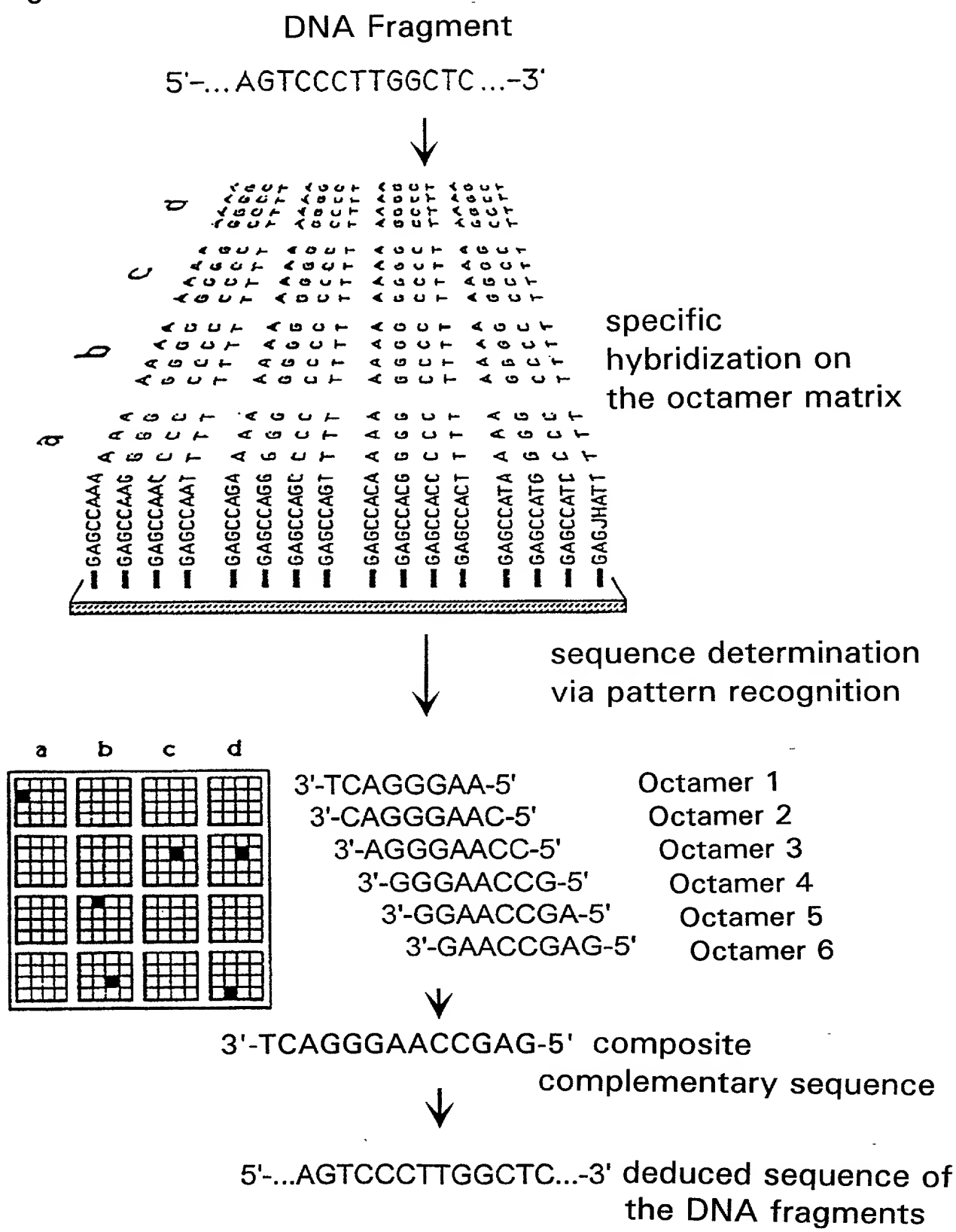
52. The method according to one of claims 50 or 51, wherein the photoinducibly redox-active moiety's oxidized electron-donor molecule or reduced electron-acceptor molecule resulting from irradiation with light of a specific or any given wavelength is rereduced or reoxidized by a suitable free redox-active substance not bound to, but in contact with the nucleic acid oligomer, i.e. the oxidized electron-donor molecule or reduced electron-acceptor molecule is restored to the state it was originally in prior to light irradiation.

53. The method of electrochemical detection according to claim 48 or 49, characterized in that the electrochemical detection is facilitated by a free redox-active substance that effectuates a chemically induced charge transfer to the redox-active moiety.

54. The method according to claim 52 or 53, wherein the free redox-active substance not bound to but in contact with the nucleic acid oligomer is selectively oxidizable and reducible at a potential φ, where φ satisfies the condition $2.0\ V \geq \varphi \geq -2.0\ V$, measured against normal hydrogen electrode.

55. The method according to one of claims 52 through 54, wherein the free redox-active substance not bound to but in contact with the nucleic acid oligomer is a free quinone, a free hexacyanoferrate(II) complex, a free sodium ascorbate, a free Ru(II)hexamine complex, or a free redox-active protein, especially a free cytochrome.

Fig. 1


DNA Fragment
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specific hybridization on the octamer matrix
sequence determination via pattern recognition
a b c d
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3'-CAGGGAAC-5' Octamer 2
3'-AGGGAACC-5' Octamer 3
3'-GGGAACCG-5' Octamer 4
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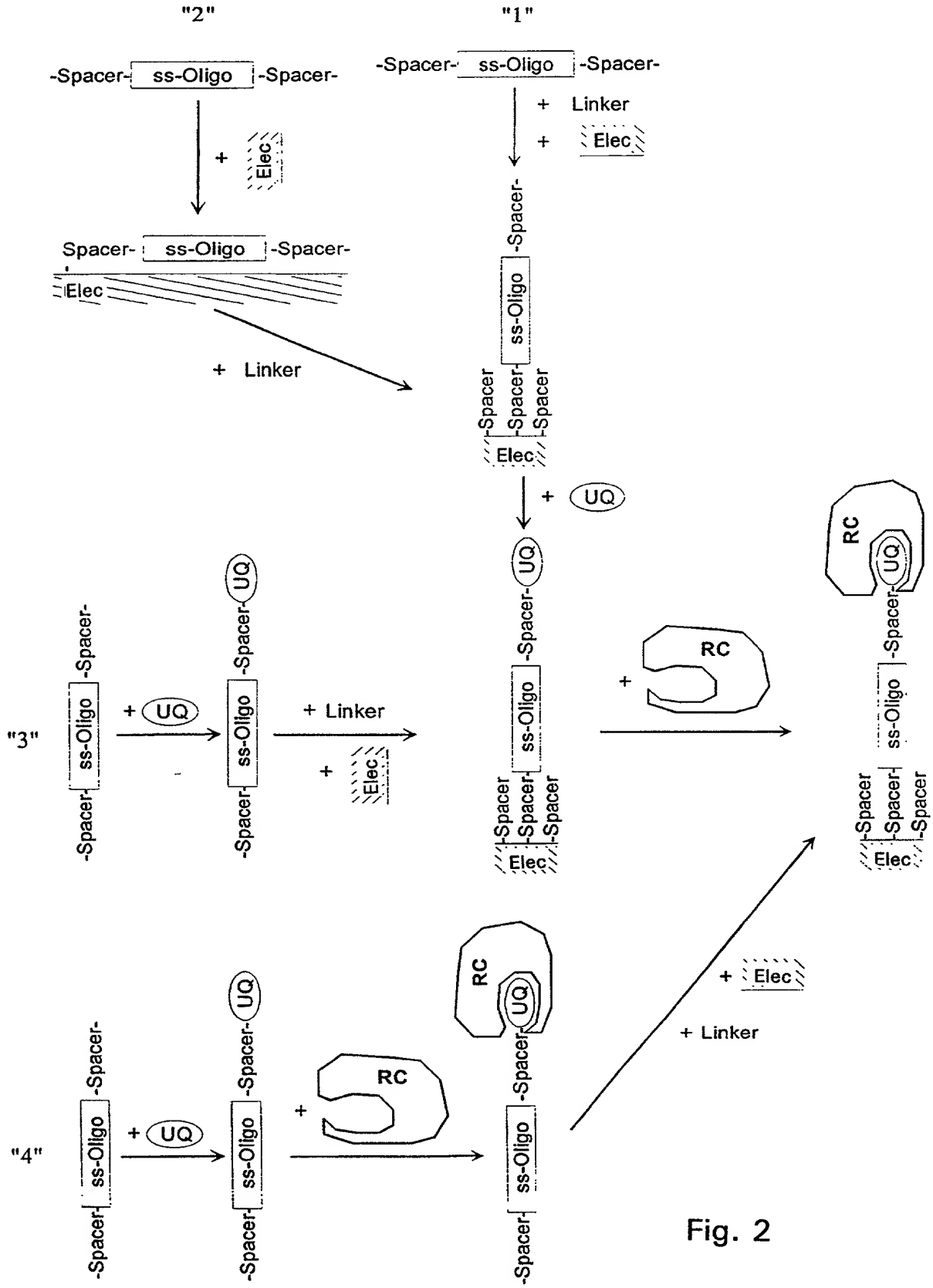
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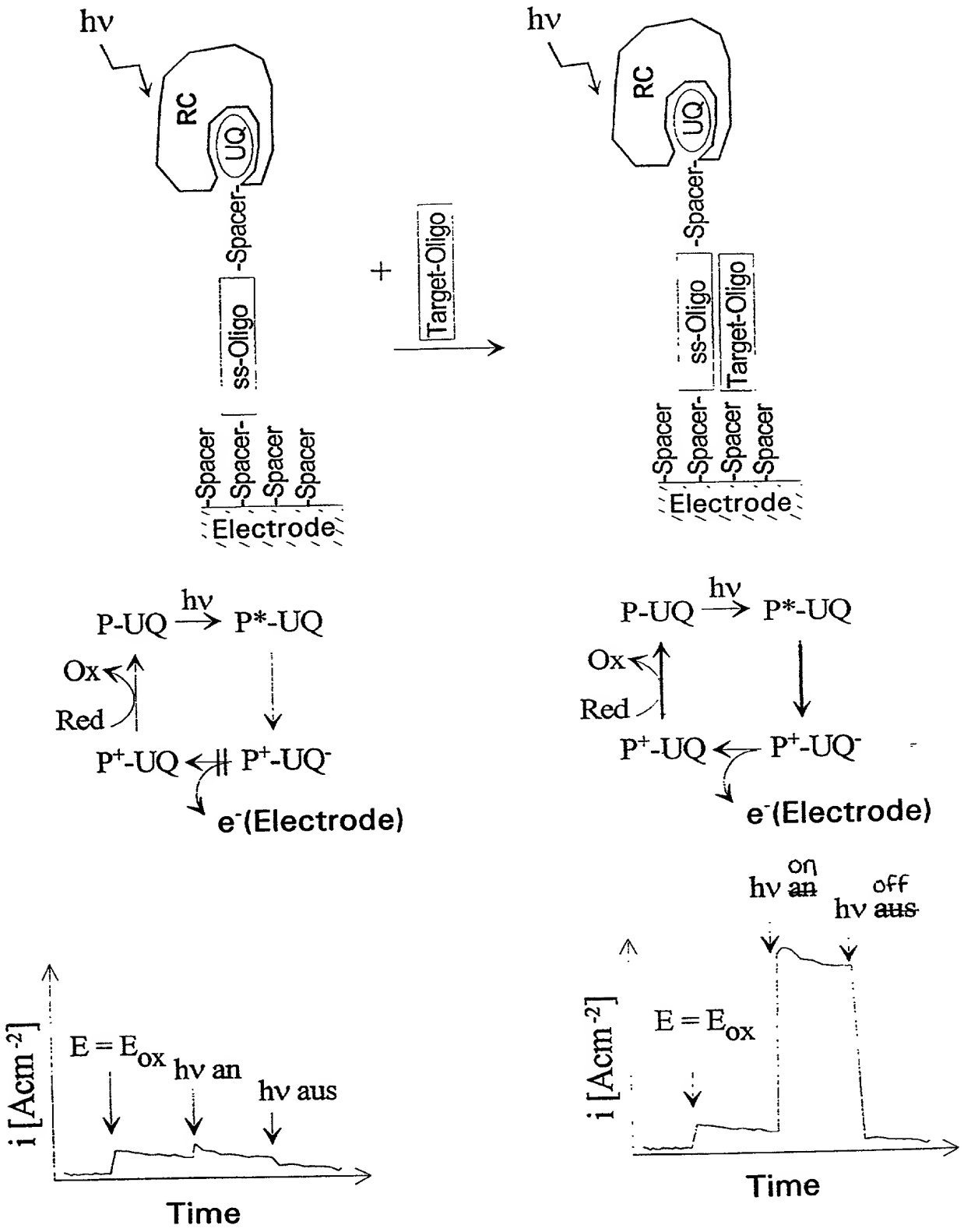
Spacer
+ UQ
UQ
RC
ss-Oligo

Fig. 2

Fig. 3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hν
P*-UQ
Ox
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Time
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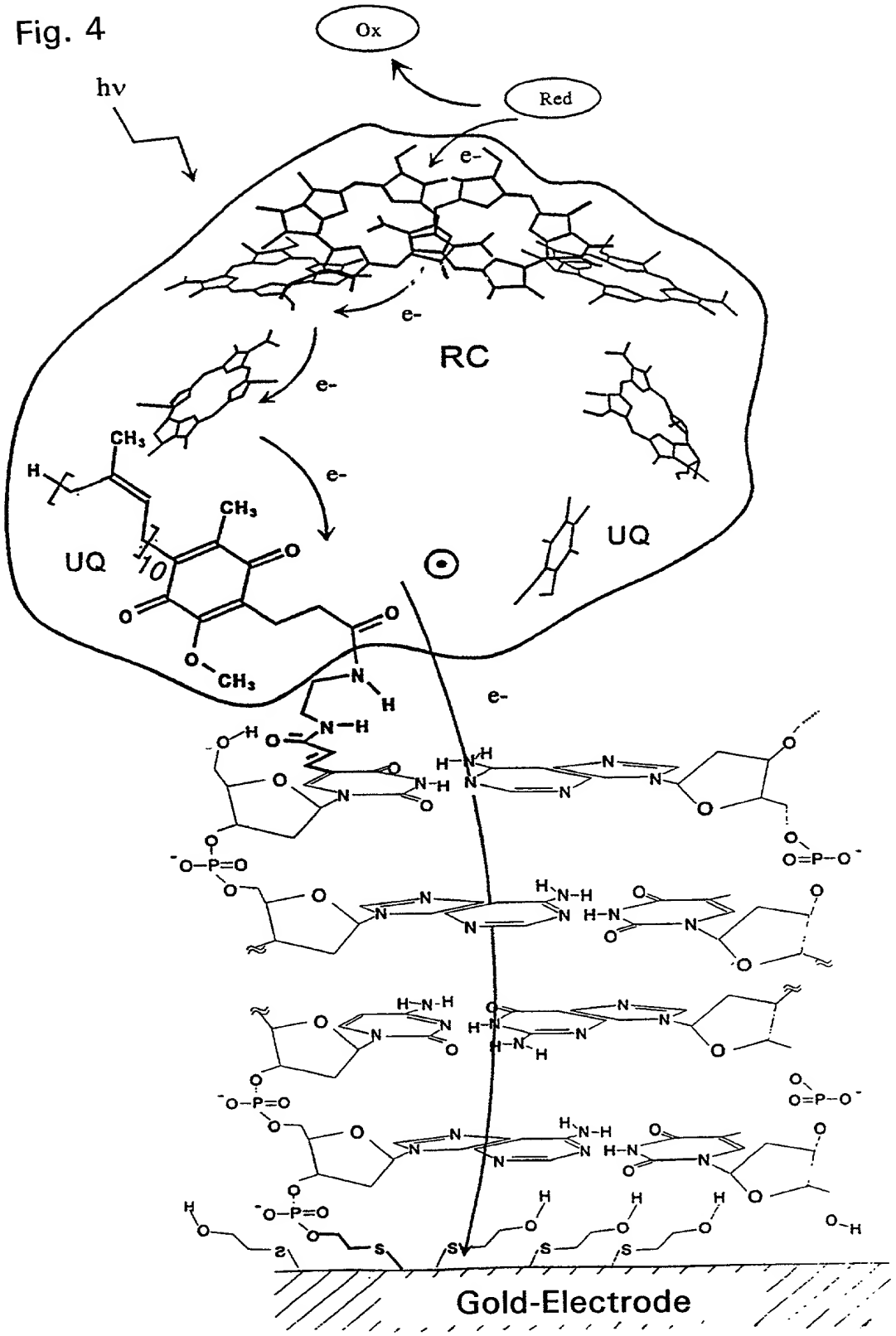
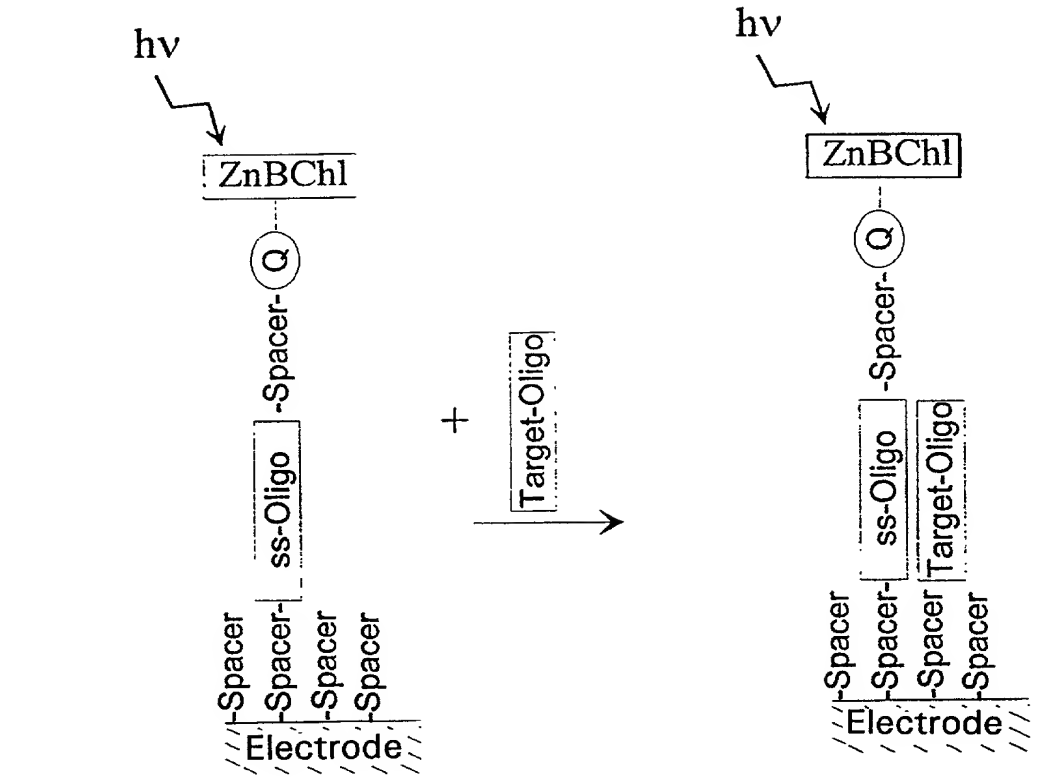
off

Fig. 4

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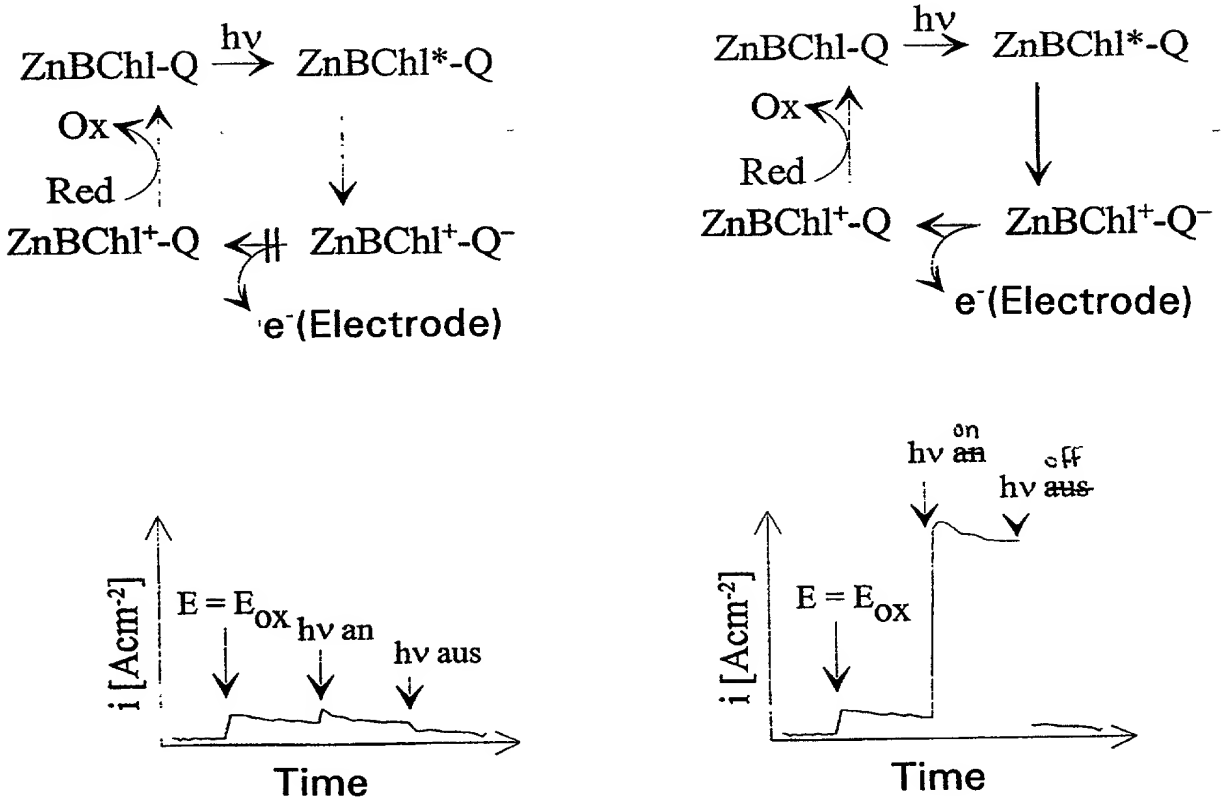
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hν
ZnBChl*-Q
Ox
Red
ZnBChl+-Q
ZnBChl+-Q-
e-(Electrode)

i [Acm-2]
E = Eox
hν an
hν aus
Time
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on
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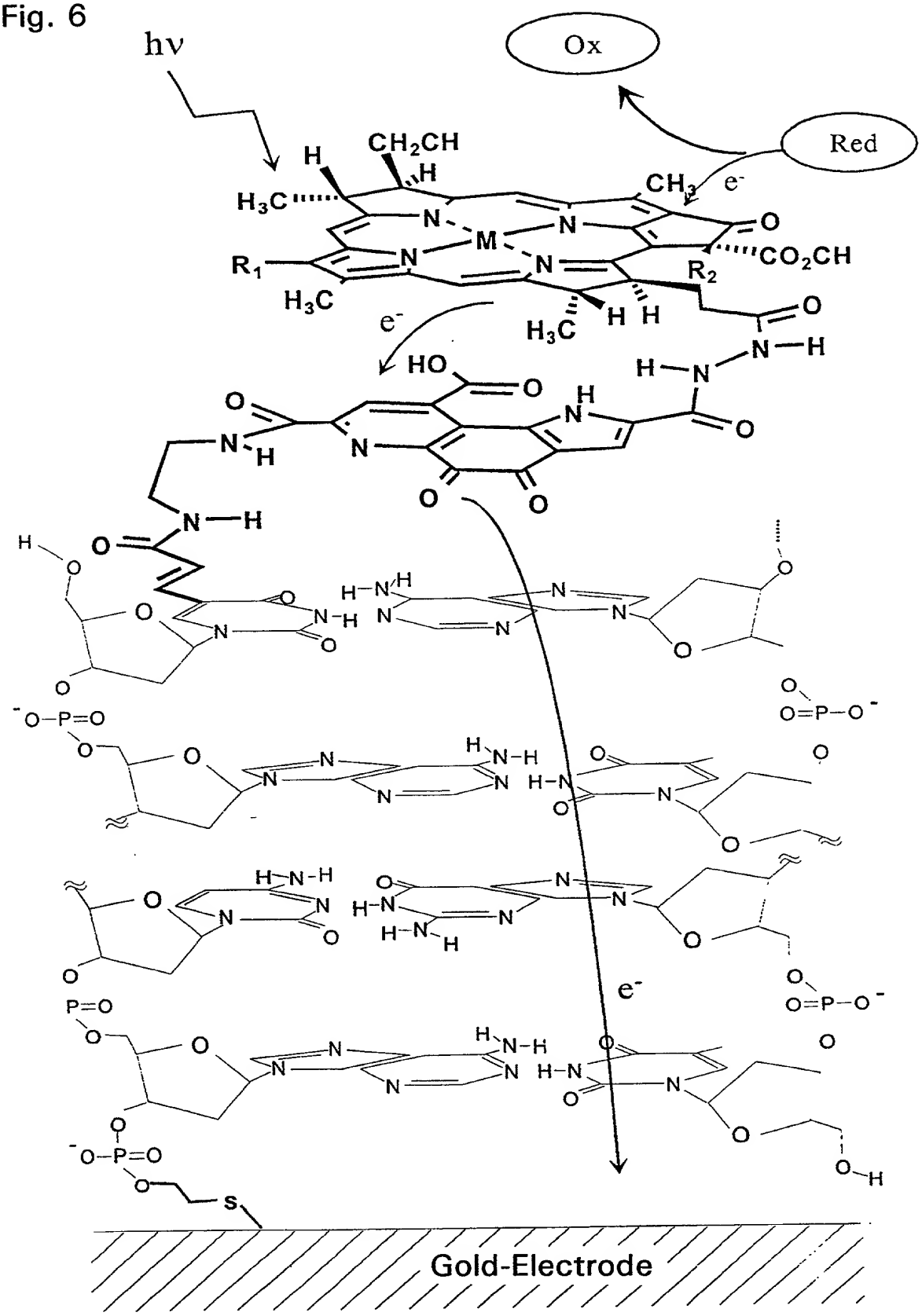
off
i [Acm-2]
E = Eox
Time

Fig. 5

Fig. 6



IMPORTANT NOTICE RE
DUTY OF CANDOR AND GOOD FAITH

The Duty of Disclosure requirements of Section 1.56(a), of Title 27 of the Code of Federal Regulations, are as follows:

A duty of candor and good faith toward the Patent and Trademark Office rests on the inventor, on each attorney or agent who prepares or prosecutes the application, and on every other individual who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application. All such individuals have a duty to disclose to the Patent Office all information they are aware of which is known to be material to patentability of the application. Such information is material where there is a substantial likelihood that a reasonable examiner would consider it important in deciding whether to allow the application to issue as a patent. The duty is commensurate with the degree of involvement in the preparation or prosecution of the application.

By virtue of this regulation, each inventor executing the Declaration for the filing of a patent application acknowledges his/her duty to disclose information of which he/she is aware and which may be material to the examination of the application.

Inherent in this is the duty to disclose any knowledge or belief that the invention:

(a) was ever known or used in the United States of America before his/her invention thereof;

(b) was patented or described in any printed publication in any country before his/her invention thereof or more than one year prior to the actual filing date of the United States patent application;

(c) was in public use or on sale in the United States more than one (1) year prior to the actual filing date of the United States patent application; or

(d) has been patented or made the subject of inventor's certificate issued before the actual filing date of the United States patent application in any country foreign to the United States on an application filed by him/her or his/her legal representative(s) or assign(s) more than twelve (12) months before the actual filing date in the United States.

NOTE: The "Information" concerned includes, but is not limited to, all published applications and patents, including applicant(s) and assignee(s) own, United States or foreign application(s) and patent(s), as well as any other pertinent prior art known, or which becomes known, to the inventor or his/her representative(s). Where English language equivalents of foreign language documents are known, they should be identified and, when possible, copies supplied. Failure to comply with this requirement may result in a patent issued on the application being held invalid even if the known prior art which is not supplied is material to only one claim of that patent.

If there is any doubt concerning whether or not a citation is "material" to patentability of the application, it is better to err on the side of safety and disclose such art to the United States Patent Office.

PATKRI P02AUS FRI062/01

COMBINED DECLARATION AND POWER OF ATTORNEY

(Original, Design, National Stage of PCT, Supplemental)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type: (check one applicable item below)

- ☐ original
- ☐ design
- ☐ supplemental
- ■ National Stage of PCT
- ☐ divisional (see added page)
- ☐ continuation (see added page)
- ☐ continuation-in-part (see added page)

INVENTORSHIP IDENTIFICATION

My/our residence, post office address and citizenship is/are as stated below next to my/our name. I/We believe that the named inventor or inventors listed below is/are the original and first inventor or inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

METHOD FOR ELECTROCHEMICALLY DETECTING NUCLEIC ACID-OLIGOMER HYBRIDISATION EVENTS

SPECIFICATION IDENTIFICATION

The specification of which: (complete (a), (b) or (c))

(a) ☐ is attached hereto.

(b) ☐ was filed on _____ as
☐ Serial No. _____ or
☐ Express Mail No _____ as Serial No. (not yet known) and was amended on _____(if applicable).

(c) ■ was described and claimed in PCT International Application No. PCT/EP00/00084 filed on January 7, 2000 and as amended under PCT Article 19 on _____ (if any).

(d) ☐ amended on _____

POWER OF ATTORNEY

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (list name(s) and registration number(s))

Anthony G. M. Davis	Registration No 27,868
Michael J. Bujold	Registration No. 32,018
Scott A. Daniels	Registration No. 42,462

(3)

☐ Attached as part of this Declaration and Power of Attorney is the authorization of the above-named attorney(s) to accept and follow instructions from my representative(s).

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Direct Telefaxes to:
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ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I/we have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent Office all information which is known to be material to patentability of this application as defined in § 1.56 of Title 37 of the Code of Federal Regulations.

PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me/us on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

COUNTRY	APPLICATION NO.	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 37 USC 119
Germany	199 01 761.1	January 18, 1999	■YES □NO
Germany	199 26 457.0	April 29, 1999	■YES □NO
			□YES □NO
			□YES □NO
			□YES □NO

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

□ I hereby claim the benefit, under 35 U.S.C. 119(e), of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YY)	□ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

DECLARATION

I hereby declare that all statements made herein of my/our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

Full name of **sole** or first joint inventor: Gerhard HARTWICH

Inventor's signature: G. Hartwich Date: 25.06.01

Residence: Nibelungenstr. 10, D 80639 München DEX

Post Office Address: Same as above Country of Citizenship: Germany